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Investigation on aroma active photooxidative degradation products originating from dimethyl pentyl furan fatty acids in green tea and dried green herbs

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Für meine Eltern
In Liebe und Dankbarkeit

Neue Ideen sind nur durch ihre Ungewohnheit schwer verständlich.

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I ABBREVIATIONS

A	area
AEDA	aroma extract dilution analysis
ASE	accelerated solvent extraction
BHT	2,6-di-tert-butyl-p-hydroxy-toluene
CHARM	combined hedonic and response measurement
DiMeF	dimethyl furan fatty acid(s)
eV	electron volt
FD	flavour dilution
FFA	furan fatty acid(s)
FID	flame ionisation detector
GC-O	gas chromatography-olfactometry
HMND	3-hydroxy-3-methyl-2,4-nonanedione
ID	inner diameter
ISTD	internal standard
MeF	monomethyl furan fatty acid(s)
MFD	meat flavour deterioration
MND	3-methyl-2,4-nonanedione
OAV	odour activity value
OC	on column

PE	polyethylene
r	correlation coefficient
R_{FID}	peak area ratio, based on FID data
RI	retention index
R_{MS}	peak area ratio, based on MS data
$R_{MS/MS}$	peak area ratio, based on characteristic product ion
SDE	simultaneous distillation solvent extraction
SIDA	stable isotope dilution assay
TIC	total ion current
WOF	warmed over flavour

II SUMMARY

In the present work furan fatty acids (FFA) and their aroma active photooxidative degradation products were investigated in dried green plant material used as foodstuff. Green tea, tarragon, basil, savory, chervil, dill, chive, onion and leek were taken as examples.

A new method using ion trap GC-MS/MS was developed for the analysis of FFA in food. The application of the GC-MS/MS technique allowed a direct, fast and sensitive analysis of FFA in the methyl ester extract of plant lipids. 12,15-Epoxy-13,14-dimethyl-eicosa-12,14-dienoic acid (DiMeF(11,5)) and 10,13-epoxy-11,12-dimethyl-octadeca-10,12-dienoic acid (DiMeF(9,5)) were the most abundant FFA found in the investigated samples. The FFA contents were in the range of a few μg/g dry matter to more than 100 μg/g. To our knowledge, the occurrence of different classes of FFA in tarragon, basil, savory, chervil, dill, onion and leek is reported for the first time in the present work.

For the isolation of the volatile compounds from the samples a micro-SDE method was adapted and critically assessed. The isolation procedure by micro-SDE was shown to be suitable with respect to artefact formation, recovery and reproducibility. The reproducibility of the method was assessed by using three internal standards. Concise results could be obtained without performing replicate analyses.

The stability of the two diones 3-methyl-2,4-nonanedione (MND) and 2,3-octanedione under photooxidative conditions was investigated in model experiments. Among the light induced oxidation products of MND the five aroma compounds 2,3-butanedione, 2,3-octanedione, acetic acid, hexanoic acid and 3-hydroxy-3-methyl-2,4-nonanedione (HMND) were identified. HMND was the main oxidation product. The odour can be described as rubbery, earthy and plastic-like. This aroma compound was identified for the first time in this work. Pentanal, acetic acid and hexanoic acid were the aroma active

oxidation products derived from 2,3-octanedione. Based on the results of the model experiments, other pathways for the formation of FFA photooxidative degradation products than the ones described in the literature have to be taken into account.

Light exposure experiments with green tea, dried herbs and vegetables were carried out to investigate the photooxidative degradation products of FFA in complex food systems. In green tea the formation rate of the aroma compounds during a long period of light exposure (20 or 25 days) in air or in oxygen atmosphere was investigated. 2,3-Butanedione showed only slight changes. The formation of MND and HMND showed a maximum after two days of light exposure, followed by a constant slight decrease. Pentanal, 2,3-octanedione, 2,3-dimethylnona-2,4-dien-4-olide (bovolide) and 2,3-dimethylnon-2-en-4-olide (dihydrobovolide) increased continuously during the time of light exposure. The major increase in the amounts of these aroma compounds occurred during the first two days. The formation of the seven aroma compounds was in accordance with the decrease of the two main pentyl DiMeF in the green tea during light exposure. After two days of light exposure, approximately 50 % of the pentyl DiMeF had reacted; after 20 days approximately 10 % of the initial amount were still present.
The investigated herbs (tarragon, basil, savory, chervil, dill and chive) as well as leek and onion showed to be differently susceptible to light exposure. The amounts of pentanal, 2,3-butanedione, 2,3-octanedione, bovolide and dihydrobovolide increased during the time of light exposure. MND and HMND were only detected in tarragon, chervil, dill, chive and leek after light exposure.

III ZUSAMMENFASSUNG

In der vorliegenden Arbeit wurden Furanfettsäuren (FFA) und deren aromaaktive, photooxidativ gebildete Abbauprodukte in grünem getrockneten Pflanzenmaterial, das als Lebensmittel verwendet wird, untersucht. Als Beispiele wurden Grüntee, Estragon, Basilikum, Bohnenkraut, Kerbel, Dill, Schnittlauch, Zwiebel und Lauch gewählt.

Für die Analyse der FFA in den gewählten Proben wurde eine Methode mit Ion Trap GC-MS/MS entwickelt. Die Anwendung der GC-MS/MS Technik erlaubte eine direkte, schnelle und empfindliche Analyse der FFA im Methylesterextrakt der Pflanzenlipide. Die zwei am häufigsten in den Proben vorkommenden FFA waren die 12,15-Epoxy-13,14-dimethyleicosa-12,14-diensäure (DiMeF(11,5)) und die 10,13-Epoxy-11,12-dimethyloctadeca-10,12-diensäure (DiMeF(9,5)). Die Furanfettsäurengehalte lagen zwischen einigen μg/g Trockensubstanz bis über 100 μg/g. Nach unserem Wissen wird hier zum ersten Mal über ein Vorkommen von verschiedenen Furanfettsäureklassen in Estragon, Basilikum, Bohnenkraut, Dill, Zwiebel und Lauch berichtet.

Für die Isolierung der flüchtigen Stoffe aus den Proben wurde eine Mikro-SDE angepasst und kritisch beurteilt. Die Isolierung mittels Mikro-SDE erwies sich bezüglich Artefaktbildung, Wiederfindung und Reproduzierbarkeit als geeignet. Die Reproduzierbarkeit der Methode wurde durch Anwendung von drei internen Standards überprüft. Damit konnten ohne Mehrfachanalysen präzise Resultate erhalten werden.

Die Stabilität der beiden Dione 3-Methyl-2,4-nonandion (MND) und 2,3-Octandion unter photooxidativen Bedingungen wurde in Modellexperimenten untersucht. Unter den lichtinduzierten Oxidationsprodukten von MND wurden die fünf Aromastoffe 2,3-Butandion, 2,3-Octandion, Essigsäure, Hexansäure und 3-Hydroxy-3-methyl-2,4-nonandion (HMND) identifiziert. HMND war das Hauptoxidationsprodukt. Der Geruch von HMND lässt sich als gummiartig, erdig und plastikartig beschreiben. Dieser Aromastoff wurde in dieser Arbeit zum ersten Mal identifiziert. Pentanal, Essigsäure

und Hexansäure gehörten zu den aromaaktiven Oxidationsprodukten von 2,3-Octandion. Basierend auf den Resultaten aus diesen Modellexperimenten müssen andere Bildungswege für die photooxidativen Abbauprodukte der Furanfettsäuren in Betracht gezogen werden als in der Literatur beschrieben.

Mit Hilfe von Belichtungsexperimenten mit Grüntee, getrockneten Kräutern und getrocknetem Gemüse wurde die Bildung der photooxidativen Abbauprodukte der FFA im komplexen System Lebensmittel untersucht. In Grüntee wurde die Bildungsrate der Aromastoffe während einer längeren Lichtexposition (20 oder 25 Tage) an der Luft oder in Sauerstoffatmosphäre ermittelt. 2,3-Butandion zeigte lediglich geringe Veränderungen. MND und HMND zeigten ein Maximun nach zweitägiger Belichtungsdauer und danach eine konstante leichte Abnahme. Pentanal, 2,3-Octandion, 2,3-Dimethylnona-2,4-dien-4-olid (Bovolid) und 2,3-Dimethylnon-2-en-4-olid (Dihydrobovolid) nahmen während der ganzen Belichtungsdauer zu, wobei die Hauptmengen während den ersten zwei Tagen gebildet wurden. Die Bildung der sieben Aromastoffe konnte in Beziehung mit der Abnahme der beiden Hauptfuranfettsäuren in Grüntee während der Belichtung gebracht werden. Nach zwei Tagen Belichtung hatten ca. 50 % der Dimethylpentylfuranfettsäuren reagiert; ungefähr 10 % der Anfangsmenge waren nach 20 Tagen Belichtung immer noch vorhanden.
Die untersuchten Kräuter (Estragon, Basilikum, Bohnenkraut, Kerbel, Dill und Schnittlauch) sowie Lauch und Zwiebeln waren unterschiedlich lichtanfällig. Die Menge an Pentanal, 2,3-Butandion, 2,3-Octandion, Bovolid und Dihydrobovolid nahm generell während der Belichtungszeit zu. MND und HMND konnten nach der Belichtung nur in Estragon, Kerbel, Dill, Schnittlauch und Lauch nachgewiesen werden.

1 INTRODUCTION

Furan fatty acids (FFA) are minor components of the lipid fraction and occur widely in different plants, vegetable oils, seafood and mammals. It is assumed that FFA are common constituents of plants and accumulate in animal tissue through the food chain (*Hannemann et al.*, 1989). Recently, this special class of lipid compounds gained in importance with the identification of FFA as precursors of light induced aroma compounds (*Guth and Grosch*, 1991; *Pompizzi et al.*, 2000). Some of the aroma active FFA photooxidative degradation products are supposed to contribute significantly to the flavour of soya-bean oil *(Guth and Grosch,* 1989), butter and butter oil (*Grosch et al.,* 1992), green tea (*Guth and Grosch,* 1993a), dry parsley (*Masanetz and Grosch,* 1998), dry spinach (*Masanetz et al.,* 1998) and anchovy (*Triqui and Reineccius*, 1995a, 1995b).

The present investigation intended to contribute to an extended insight of FFA and their aroma active photooxidative degradation products in dried green plant material used as foodstuff. The studies were undertaken with green tea, dried green herbs and some selected vegetables. The investigation focused on:

- Analysis of FFA
- Light exposure experiments to induce the formation of FFA photooxidative degradation products
- Analysis of the aroma active FFA photooxidative degradation products in the selected samples

Established procedures to analyse FFA in biological samples are all time-consuming or require uncommon equipment. Therefore a method had to be developed for an easier practicable and faster analysis of FFA in the selected samples.

In order to study the aroma active photooxidative degradation products of FFA it is necessary to isolate the volatile substances from the nonvolatile plant material. The sample preparation is the most critical step in the entire analytical process of the investigation of volatile compounds (*Schreier*, 1984). Various isolation techniques have been developed (see e.g. in *Schreier*, 1984 or *Maarse and Grosch*, 1996). In this part of the investigation it was therefore very important to choose and assess a suitable method for the isolation of aroma compounds and to carefully adapt it to the corresponding problem.

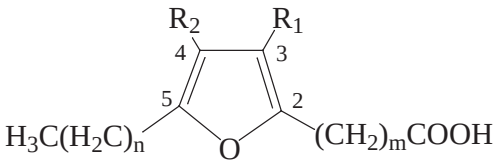
Light exposure experiments in model systems were conducted in order to complement and to extend the understanding of the formation and possible pathways of products derived from FFA under photooxidative conditions. Finally light exposure experiments with green tea and dried herbs and vegetables should result in a better comprehension of light induced processes in the complex food system.

2 LITERATURE REVIEW

2.1 Furan fatty acids

2.1.1 Structure and nomenclature

Furan fatty acids (FFA) (Fig. 1) are characterised by the presence of a di-, tri- or tetrasubstituted furan ring in the molecule. They differ in the degree of methyl substitution at position 3 and 4 and in the length of the alkyl carboxyl chain and the alkyl chain. The most common members of the dimethyl substituted FFA (DiMeF) are the propyl FFA (bearing a terminal propyl group) and the pentyl FFA (bearing a terminal pentyl group).



R_1 & R_2 = H
R_1 & R_2 = CH_3
R_1 = CH_3 & R_2 = H

n = 2: propyl FFA
n = 4: pentyl FFA

Figure 1: Structure of furan fatty acids

For the nomenclature two different abbreviation systems are used. *Glass et al.* (1975) named the FFA F1, F2,... based on the sequence of elution in the GC. *Rahn et al.* (1981) suggested a self-explanatory abbreviation nomenclature. The FFA are termed as F(m+1, n+1), MeF(m+1, n+1) or DiMeF(m+1, n+1). F stands for the furan ring, the prefix Me or DiMe for a mono- or dimethyl substitution at position 3 or at position 3 and 4, respectively. The affix (m+1, n+1) indicates the chain lengths of the alkyl carboxyl group at position 2 and the alkyl group at position 5. In this thesis, the abbreviated form

by *Rahn et al.* (1981) will be used. Tab. 1 gives an overview of the nomenclature and the abbreviations of the most common FFA.

Table 1: Nomenclature of furan fatty acids

systematic nomenclature	*Glass et al.* (1975)	*Rahn et al.* (1981)	R_1	R_2	m	n
10,13-epoxy-11,12-dimethyl-hexadeca-10,12-dienoic acid	F1	diMeF(9,3)	CH_3	CH_3	8	2
10,13-epoxy-11-methylocta-deca-10,12-dienoic acid	F2	MeF(9,5)	CH_3	H	8	4
10,13-epoxy-11,12-dimethyl-octadeca-10,12-dienoic acid	F3	diMeF(9,5)	CH_3	CH_3	8	4
12,15-epoxy-13,14-dimethyl-octadeca-12,14-dienoic acid	F4	diMeF(11,3)	CH_3	CH_3	10	2
12,15-epoxy-13-methyl-eicosa-12,14-dienoic acid	F5	MeF(11,5)	CH_3	H	10	4
12,15-epoxy-13,14-dimethyl-eicosa-12,14-dienoic acid	F6	diMeF(11,5)	CH_3	CH_3	10	4
14,17-epoxy-15-methyl-docosa-14,16-dienoic acid	F7	MeF(13,5)	CH_3	H	12	4
14,17-epoxy-15,16-dimethyl-docosa-14,16-dienoic acid	F8	DiMeF(13,5)	CH_3	CH_3	12	4

In nature, DiMeF are predominant, whereas monomethyl substituted FFA (MeF) are generally present in significantly lower concentrations. Natural occurrence of non methyl substituted FFA is uncertain. *Yurawecz et al.* (1995) determined the non methyl substituted FFA as oxidation products of conjugated linoleic acids in model experiments. This class of FFA will not be discussed in detail in this work.

2.1.2 Occurrence

The analysis of different lipid fractions have revealed FFA to be constituents of cholesteryl esters, triglycerides and phospholipids, depending on the samples investigated. In freshwater fish for example, the FFA occurred in triglycerides and esterified to cholesterol (*Glass et al.*, 1974), whereas in human blood plasma and bovine blood plasma (*Puchta et al.*, 1988) and in sugar cane (*Saccharum spec*.) cells (*Scheinkönig and Spiteller*, 1993) they mainly occurred in phospholipids.

F(8,6) was the first fatty acid of the furanoid type to be reported. *Morris et al.* (1966) claimed that F(8,6) occurs in the *Exocarpus* seed oil. This result could not be confirmed by *Gunstone et al.* (1978) who supposed this hexyl FFA to be an artefact from an associated oxygenated acetylenic fatty acid. Investigations by *Glass et al.* (1974, 1975, 1977) showed a wide occurrence of different FFA in freshwater fish. Lower percentages than in freshwater fish have been found also in various species of marine fish by *Gunstone et al*. (1976, 1978), *Gunstone and Wijesundera* (1978), *Scrimgeour* (1977), *Yoshioka* (1981), *Ota and Takagi* (1989a, 1989b, 1990, 1991, 1992) and *Itabashi et al.* (1994, 1995). *Wahl et al.* (1994) and *Wahl* (1998) investigated the occurrence of FFA in fish oil and fish oil compounds. Low levels of FFA have been reported to occur in other aquatic species as well, such as in the tissues of crayfish (*Okajima et al.*, 1984; *Ishii et al.*, 1988a, 1989a; *Dembitsky and Rezanka*, 1996), octopus (*Gunstone et al.*, 1978), sea squirt (*Yoshioka*, 1981), scallops (*Yoshioka*, 1981; *Dembitsky and Rezanka*, 1996), soft corals (*Groweiss and Kashman*, 1978) and sponges *(Ciminiello et al.*, 1991; *Prinsep et al.*, 1994). The list can be expanded to amphibia such as bullfrog, and reptiles such as turtles as examples (*Ishii et al.*, 1988a).

The occurrence of FFA in plants seems to be of greater importance. In the latex of the rubber tree *Hevea brasiliensis* MeF(9,5) has been identified as the main component of the lipid fraction (*Hasma and Subramaniam*, 1978; *Lie Ken Jie and Sinha*, 1980). Different FFA were detected as minor components in numerous other plants (*Hannemann et al.*, 1989), in yeast (*Hannemann et al.*, 1989) and in algae (*Kazlauskas et al.*, 1982; *Hannemann et al.*, 1989; *Batna et al.*, 1993; *Itabashi et al.*, 1995). The levels were higher in photosynthetic tissues than in the other parts of the plants. Recently, the occurrence of FFA in green tea (*Guth and Grosch*, 1993a), dried spinach (*Masanetz et al.*, 1998) and dried parsley (*Masanetz and Grosch*, 1998) was reported.

FFA have been identified as minor components in blood, liver, muscle and lipid tissue and in milk of rats (*Gorst-Allman et al.,* 1988), cattle (*Jandke and Spiteller,* 1988; *Schödel and Spiteller,* 1987; *Puchta et al.,* 1988; *Guth and Grosch,* 1992) and humans (*Puchta et al.,* 1988; *Puchta and Spiteller,* 1988; *Wahl et al.,* 1995). This indicates an ubiquitous distribution of FFA in nature. However, it remains an open question if FFA are synthesised *de novo* or introduced by the diet in mammals.

As for the origin of FFA in fish, recent findings indicate that they possibly arise not only from marine plants but also from intestinal bacteria of fishes. *Shirasaka et al.* (1995, 1997) investigated MeF in marine bacteria such as *Shewanella putrefaciens* and *Pseudomonas fluorescens*. Very recently, MeF was also identified in a *Bacillus* sp. (*Carballeira et al.* 2000). In Tab. 2 all FFA detected up to now are listed in groups.

Table 2: Occurrence of furan fatty acids

FFA	first detected in	reference
propyl		
MeF(9,3)	brown alga	*Kazlauskas et al.* (1982)
MeF(11,3)	salmon roe	*Ishii et al.* (1988b)
DiMeF(7,3)	crayfish	*Ishii et al.* (1988a)
DiMeF(9,3)	northern pike	*Glass et al.* (1974)
DiMeF(11,3)	northern pike	*Glass et al.* (1974)
DiMeF(13,3)	different marine and freshwater fish	*Gunstone et al*. (1978)
DiMeF(15,3)	fish oil	*Wahl et al.* (1994)
butyl		
MeF(9,4)	crayfish	*Ishii et al.* (1988a)
DiMeF(9,4)	crayfish	*Ishii et al.* (1988a)
DiMeF(11,4)	salmon roe	*Ishii et al.* (1988b)

Table 2: Occurrence of furan fatty acids (cont.)

FFA	first detected in	reference
pentyl		
MeF(3,5)	crayfish	*Ishii et al.* (1988a)
MeF(5,5)	butter and butter oil	*Guth and Grosch* (1992)
MeF(7,5)	crayfish	*Ishii et al.* (1988a)
MeF(9,5)	northern pike	*Glass et al.* (1974)
MeF(11,5)	northern pike	*Glass et al.* (1974)
MeF(13,5)	northern pike	*Glass et al.* (1974)
MeF(15,5)	crayfish	*Ishii et al.* (1988a)
DiMeF(3,5)	soft corals	*Groweiss and Kashman* (1978)
DiMeF(5,5)	crayfish	*Ishii et al.* (1988a)
DiMeF(7,5)	crayfish	*Okajima et al.* (1984)
DiMeF(8,5)	crayfish	*Ishii et al.* (1988a)
DiMeF(9,5)	northern pike	*Glass et al.* (1974)
DiMeF(10,5)	crayfish	*Ishii et al.* (1988a)
DiMeF(11,5)	northern pike	*Glass et al.* (1974)
DiMeF(12,5)	crayfish	*Ishii et al.* (1988a)
DiMeF(13,5)	northern pike	*Glass et al.* (1974)
DiMeF(15,5)	mollusc	*Dembitsky and Rezanka* (1996)
hexyl		
F(8,6)	*Exocarpus* seed oil	*Morris et al.* (1966)
MeF(9,6)	crayfish	*Ishii et al.* (1988a)

Table 2: Occurrence of furan fatty acids (cont.)

FFA	first detected in	reference
DiMeF(7,6)	crayfish	*Ishii et al.* (1988a)
DiMeF(9,6)	crayfish	*Ishii et al.* (1988a)
DiMeF(11,6)	crayfish	*Ishii et al.* (1988a)
heptyl		
MeF(5,7)	crayfish	*Ishii et al.* (1988a)
MeF(11,7)	crayfish	*Ishii et al.* (1988a)
DiMeF(7,7)	crayfish	*Ishii et al.* (1988a)
miscellaneous		
MeF(9,1)	brown alga	*Kazlauskas et al.* (1982)
F(2,14)Δ8,11,14,17	marine sponge	*Ciminiello et al.* (1991)
F(2,20)Δ9,19	marine sponge	*Ciminiello et al.* (1991)

2.1.3 Relevance

The role of the FFA in biological systems remains more or less obscure. According to *Batna and Spiteller (*1994a, 1994b) FFA could be important in plant defence through the products resulting from their oxidative degradation. Further it is assumed that FFA may serve as antioxidants because of their hydroxyl radical scavenging activity (*Ishii et al.,* 1989b; *Okada et al.*, 1990a, 1996) and their inhibitory activity on horseradish peroxidase (*Fuchs and Spiteller*, 1999). *Okada et al.* (1990b) examined an inhibitory effect on the haemolysis of erythrocytes induced by singlet oxygen and found the FFA to be effective quenchers. Additionally, FFA have been reported to have inhibitory effects on blood platelet aggregation (*Graff et al.,* 1984) and on bacterial urease activity (*Rosenblat et al.,* 1993) and to have potential antitumor activity (*Isoda et al.,* 1993). One non methyl substituted FFA (F(5,6)) also showed a toxic effect on *Tripanosoma brucei*, which causes the sleeping sickness (*Doering et al.*, 1994).

FFA are considered as precursors of some potent aroma compounds. 3-Methyl-2,4-nonanedione (MND) was the first aroma compound that was recognised to be a photooxidative degradation product of pentyl DiMeF (*Guth and Grosch,* 1991). Moreover, *Sarelse et al*. (1994) put forth the hypothesis that 2,3-dimethylnona-2,4-dien-4-olide (bovolide) and 2,3-dimethylnon-2-en-4-olide (dihydrobovolide) were formed by photooxidation of FFA. This could be confirmed experimentally by *Pompizzi et al*. (1997). The importance of pentyl DiMeF as precursors of flavour compounds was systematically investigated by *Pompizzi* (1999) in his doctoral thesis. Photooxidative and autoxidative model experiments were carried out with DiMeF(9,5) methyl ester. Among the oxidation products, eight aroma compounds could be identified, namely pentanal, 2,3-butanedione, 2,3-octanedione, MND, bovolide, dihydrobovolide, hexanoic acid and valeric acid. Model experiments with DiMeF(11,3) showed the formation of the analogous aroma compounds through oxidation: propanal, 2,3-hexanedione, 3-methyl-2,4-heptanedione, 2,3-dimethylhepta-2,4-dien-4-olide and 2,3-dimethylhept-2-en-4-olide. In the following chapters an overview of the six aroma compounds which are relevant for the present work is given.

2.2 Aroma active photooxidative degradation products of dimethyl pentyl furan fatty acids

2.2.1 3-Methyl-2,4-nonanedione

MND is a β-diketone, of which the keto and enol tautomers can easily be separated by GC using a nonpolar capillary column (*Masur et al.,* 1987). *Guth and Grosch* (1989) observed a major and a minor peak in the gas chromatogram obtained using a SE-30 capillary as stationary phase. The increase in the base-line between the two peaks was caused most likely by changes in the keto-enol equilibrium during the GC analysis.
The first odour descriptor for MND was lard-like, strawy and fruity (*Guth and Grosch,* 1989). Additional odour qualities perceived at the sniffing port were sweet (*Triqui and Reineccius,* 1995a), hay-like (*Masanetz et al.,* 1998) and green (*Kumazawa and Masuda,* 1999). Often only one of these descriptors or a combination are used in the literature. A differing odour descriptor for MND is anis-like and brackish (*Schlüter et al.,* 1996). The odour thresholds in different media are given in Tab. 3.

Table 3: Odour thresholds for MND in different media

medium	threshold	reference
air	0.007-0.014 ng/l	*Guth and Grosch* (1989)
	0.06 ng/l (keto form)	*Pompizzi* (1999)
	0.05 ng/l (enol form)	*Pompizzi* (1999)
water	0.03 µg/kg (orthonasal)	*Masanetz and Grosch* (1998)
	0.02 µg/kg (retronasal)	*Guth and Grosch* (1993a)
oil	15-30 µg/kg (orthonasal)	*Guth and Grosch* (1989)
	1.5 µg/kg (retronasal)	*Guth* (1991)
cellulose	1.5 µg/kg	*Guth and Grosch* (1993a)

Based on an aroma extract dilution analysis (AEDA), *Guth and Grosch* (1989, 1990a) identified MND as the most important odorant of soy bean oil, which had a „beany-strawy“ off-flavour after storage in daylight for 30 days. Quantification by stable isotope dilution assay (SIDA) confirmed the importance of MND in the so called reversion flavour of soy bean oil. The calculated odour activity value (OAV, ratio of the concentration of the aroma compound to its threshold) indicated that MND was the most flavour-active compound after 48 h of storage in daylight (*Guth and Grosch*, 1990b). Detection of FFA as precursors of MND in soy bean oil clarified the correlation between MND, FFA and the light-induced deterioration of soy bean oil (*Guth and Grosch*, 1991). However, findings by *Kao et al.* (1998) did not support the theory that FFA or MND contribute strongly to the reversion flavour. *Guth and Grosch* (1999) confirmed their suggestion in a letter to the editor. The potent odorants in standardised, enzymatically hydrolysed and deoiled soybean lecithins were systematically characterised by *Stephan and Steinhart* (1999a). MND was found to be an important odorant on the basis of the flavour dilution (FD) factor and combined hedonic and response measurement (CHARM) values. However, MND was also only one of a large number of odorants that are responsible for the overall lecithin aroma. This suggestion was confirmed by quantification and calculation of the nasal and retronasal OAVs (*Stephan and Steinhart*,

1999b). Though MND showed a potent retronasal OAV, the compound had no outstanding sensory characteristics in soybean lecithin. The authors therefore concluded that MND cannot be the character impact compound that is solely responsible for the main strawy and grain-like odour of soybean lecithins.

Investigations by *Grosch et al.* (1992) showed that exposure of butter and butter oil to fluorescent light changed the flavour from buttery, sweet and acidulous to green, strawy and fatty. A comparative AEDA indicated that this change was mainly due to the production of MND. This result has been completed with the identification of FFA in butter and butter oil as precursor of MND causing the off-flavour (*Guth and Grosch,* 1992).

AEDA and calculation of OAV revealed MND as one of the most significant odorants of green tea powder and brew (*Guth and Grosch,* 1993a). This result was associated to the occurrence of FFA in green tea. Already some years ago, *Horita and Hara* (1986) reported on an unknown light-produced aroma compound of green tea. The mass spectral characteristics of this compound were in agreement with those of MND. MND was detected in black tea as well, and evaluated by AEDA and calculation of OAV (*Guth and Grosch,* 1993b). Although the dione occurred in similarly high concentration as in green tea, it was not considered to be a key aroma compound since other potent aroma compounds masked the hay-like odour. Recently *Kumazawa and Masuda* (1999) identified potent odorants in Japanese green tea (Sencha) based on AEDA. MND was not found to belong to the eight most potent odour compounds. The authors mentioned that the Japanese green tea is manufactured according to a process different from the one used for Chinese green tea. *Guth and Grosch* (1993a) investigated Chinese green tea powder and assumed that the MND was formed during the processing of green tea.

Masanetz and Grosch (1998) showed by AEDA and calculation of OAV that MND was mainly responsible for the hay-like off-flavour of dried parsley, which developed during drying and storage. In these samples FFA were detected as precursors as well. Dry spinach was investigated by *Masanetz et al.* (1998). The detection of FFA and MND led to the conclusion that the hay-like off-flavour was caused by an oxidative degradation of FFA to MND. However, in extracts of cooked spinach leaves, MND was only detected in traces (*Näf and Velluz,* 2000). Depending on the concentration, a contribution to the pleasant and warm hay-cereal-flour-like and buttery, green-woody odour was not excluded. An interesting fact was the detection of (E)-3-methyl-2-nonen-4-one in the

cooked spinach leaves as a heavy, tenacious compound exhibiting a green, stalk, lovage-like note. Its structure is related to MND, and it could be a degradation product of bovolide (see chapter 2.2.3).

So far, MND has not only been detected in plants, but also in fish. *Triqui and Reineccius* (1995a, 1995b) investigated the flavour development in anchovy during ripening by means of AEDA. MND was reported to be an important flavour component. Since it was identified in anchovy just after salting, a formation following evisceration was assumed. Natural sensitisers in fish may generate singlet oxygen which may then react with FFA present in anchovy. *Schlüter et al.* (1996) used GC-O, AEDA and CHARM to analyse aroma extracts of steamed fillets of carp fed either with zooplankton or mainly with wheat. MND was perceived only in the sample fed with plankton. Based on the FD factor and the CHARM value, MND was not considered to be important. Somewhat later, MND was mentioned as a potent odorant in boiled carp fillet based on FD factors (*Schlüter et al.*, 1999). Based on the FD factor as well, MND was reported to be a potent odorant in boiled trout (*Milo and Grosch*, 1993).

Two different pathways for the photooxidative formation of MND have been described in the literature. *Guth and Grosch* (1991) proposed an ene-reaction of a pentyl DiMeF with singlet oxygen directly to a hydroperoxide. β-scission of the hydroperoxy-group and subsequent further cleavages resulted in the keto-enol form of MND and a ketene (Fig. 2). *Pompizzi et al.* (2000) suggested a cycloaddition of singlet oxygen to DiMeF(9,5) to a bicyclic furan endoperoxide. Rearrangements then resulted in the β-keto-enol ester that was transformed by hydrolysis to MND (Fig. 3).

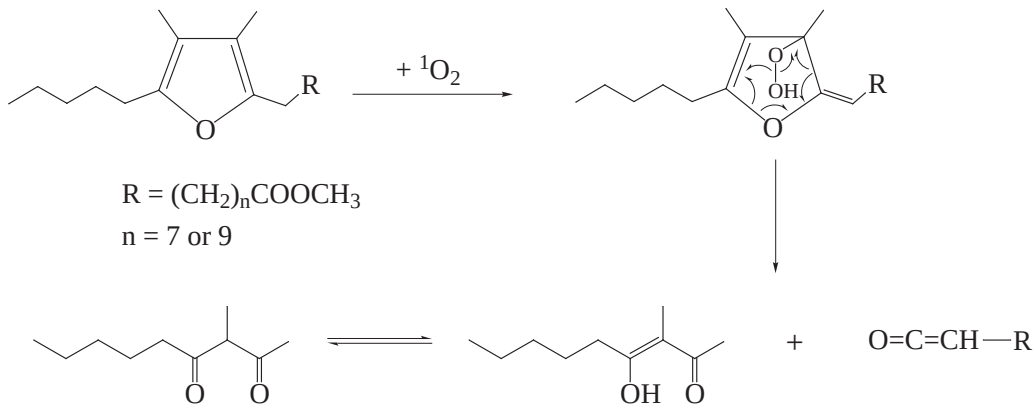


Figure 2: Proposed pathway for the photooxidative formation of MND (*Guth and Grosch,* 1991)

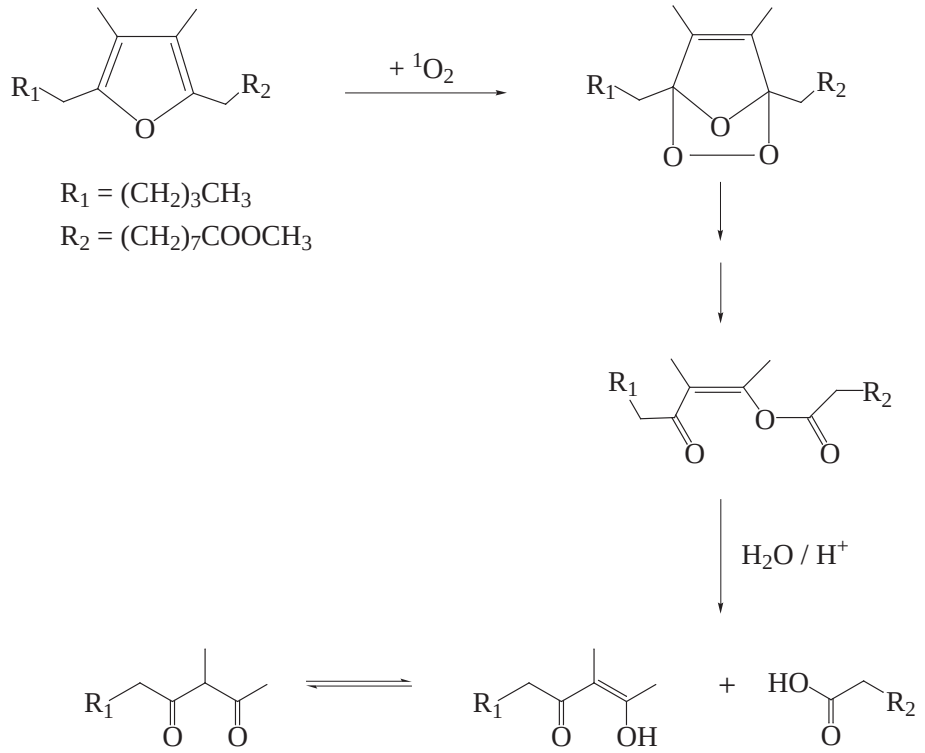


Figure 3: Proposed pathway for the photooxidative formation of MND (*Pompizzi et al.*, 2000)

2.2.2 2,3-Octanedione

2,3-Octanedione is an aroma compound which odour has been described in many different ways in the literature. *Winter et al*. (1976) used the descriptor caramel-like and sweet. The odour has also been described as sweet and fruity (*Suzuki,* 1985), fragrant and meaty (*Ames and Elmore*, 1992), fatty (*Sutherland and Ames*, 1995), green, ketoney, grassy and strawy (*Braggins,* 1996), hay-like and fruity (*Pompizzi et al.*, 2000), pungent and sour (*Hartvigsen et al.,* 2000) and mushroom-like (*Ulrich et al.,* 2001). The odour threshold in air is 0.09 ng/l (*Pompizzi,* 1999), in oil orthonasal 2.0 mg/kg (*Pompizzi,* 1999) and in water orthonasal 0.11 mg/kg (*Sigrist et al.*, 2000).

2,3-Octanedione was detected as volatile compound in tobacco (*Demole and Berthet,* 1972), dried mushroom (*Vidal et al.,* 1986), bell peppers fruits (*Matsui et al.,* 1997), apricot pureé (*Bolzoni et al.,* 1990), prickly pear juice (*Di Cesare and Nani,* 1992) and black elder flowers (*Mazza,* 2001). None of these studies contain any information of a possible contribution of 2,3-octanedione to the aroma. In heated blackberry juices (*Georgilopoulos and Gallois,* 1987) and in anise (*Kollmannsberger et al.,* 2000) the diketone was identified but recognised as a compound with no odour intensity. In potato flesh, 2,3-octanedione was identified by *Oruna-Concha et al.* (2001) as a volatile but not as a key aroma compound. 2,3-Octanedione was detected in traces in the volatile constituents of used frying oil (*Takeoka et al.,* 1996). Traces present in soybean oil and corn oil underwent quantitative changes during storage due to oxidation (*Wu and Chen,* 1992). In dried green plant material 2,3-octanedione was detected in the headspace of sun-cured fescue hay (*Mayland et al.,* 1997) and in Oolong tea (*Suxian et al.,* 1998). Additionally, *Horita and Hara* (1986) reported on an unknown volatile compound of green tea exposed to light, which mass spectral characteristics fitted to 2,3-octanedione. In cereals, 2,3-octanedione has often been described as a product of lipid degradation with no further explanation to its formation. *Pfannhauser* (1990) identified the dione in triticale treated by cooking extrusion, and *Hwang et al.* (1994) in extruded wheat flour. *Bailey et al.* (1994a) identified it in an extruded product of a mixture of whey protein concentrate with corn meal, and *Parker et al.* (2000) in extruded oat flour. *Nijssen et al.* (1996) found 2,3-octanedione in soy and unfermented soy products, and *Aaslyng et al.* (1998) in the headspace of hydrolysed soy protein. 2,3-Octanedione was also present in wheat bread crust (*Chang et al.,* 1995) and in sour dough bread (*Seitz et al.,* 1998). In

yeast extract 2,3-octanedione has been stated as an aroma compound (*Ames and Elmore*, 1992). Recently, 2,3-octanedione was detected by GC-O (CHARM) as a key compound in boiled asparagus and a contribution to the aroma was assumed (*Ulrich et al.,* 2001).

The occurrence of 2,3-octanedione in meat and meat products seems to be of much greater significance than in plants. 2,3-Octanedione was first detected in beef (roast beef) by *Liebich et al.* (1972), in mutton (cooked mutton) by *Nixon et al.* (1979), in poultry (roasted chicken) by *Noleau and Toulemonde* (1986), in chevon (cooked chevon) by *Lamikanra and Dupuy* (1990), in pork (uncured pork) by *Ramarathnam et al.* (1991a) and in venison (deer) by *Clarke et al.* (1995), who in addition reported 2,3-octanedione to be a suitable marker compound to predict the duration of frozen storage of meat. *Young et al.* (1997) investigated the influence of the diet on volatiles of lamb fat and found 2,3-octanedione to be an excellent indicator of a pasture diet. Already earlier *Suzuki and Bailey* (1985) reported on very high contents of 2,3-octanedione in the volatiles from ovine fat of animals finished on clover, compared to those of animals finished on corn. Later, *Bailey et al.* (1994b) investigated the influence of finishing diets on lamb flavour. Meat from animals finished on corn grain was milder than that of animals finished on forage, and 2,3-octanedione was one of the volatile compounds correlating with flavour strength. In beef, 2,3-octanedione was reported to be an indicator for a pasture diet (*Larick et al.,* 1987) as well. However, in a study with forage-fed goats, 2,3-octanedione was less prominent in the cooked meat (*Lamikanra and Dupuy,* 1990).

In the context of meat flavour quality, a possible correlation of 2,3-octanedione and the so called warmed-over flavour or WOF (also referred to as meat flavour deterioration, or MFD) has to be mentioned. WOF is the rapid development of an off-flavour in refrigerated cooked meat described as rancid or stale (*Tims and Watts,* 1958). Although the precise mechanisms of the formation of WOF have not yet been elucidated, the generally accepted theory states that WOF is caused by the oxidation of membrane phospholipids, catalysed by an ionic form of iron (*Pearson and Gray,* 1983; *Love,* 1987). Among some other aroma compounds, 2,3-octanedione was first proposed by *St. Angelo et al.* (1987a) as WOF marker due to its high correlation with WOF formation in beef. Further studies on WOF were performed with 2,3-octanedione as marker in cooked turkey rolls (*Wu and Sheldon,* 1988), beef (*Vercellotti et al.,* 1988), cooked chevon

(*Lamikanra and Dupuy,* 1990), beef patties stored under vacuum (*Spanier et al.,* 1992a, 1992b) and freeze-dried lean beef (*Thongwong et al.,* 1999). However, many studies on WOF exist in which 2,3-octanedione has not even been mentioned.
Some results of investigations on uncured and cured meat are noteworthy. *Ramarathnam et al.* (1991a) found 2,3-octanedione to be present in appreciable levels in uncured pork but absent in cured pork. The same result was obtained with uncured and cured beef and chicken (*Ramarathnam et al.,* 1991b). However, *Dirinck et al.* (1997) and *Ruiz et al.* (1998, 2001) detected the dione in dry-cured Iberian ham and *Timón et al.* (2001) in the subcutaneous fat from dry-cured hams. *Ruiz et al.* (1999) observed that the volatile compounds of dry-cured ham were affected by the length of the curing process; 2,3-octanedione decreased significantly during the ripening process.
With regard to meat products, 2,3-octanedione was detected in dry fermented sausages (*Berdagué et al.,* 1993; *Ansorena et al.,* 1998) and Frankfurter sausages (*Chevance and Farmer,* 1999). *Berdagué et al.* (1993) observed a significant influence of the nature of the starter culture on the formation of 2,3-octanedione in dry sausages.

2,3-Octanedione has also been detected in fish. At first, the dione was only found in some freshwater fish species but not in the surveyed saltwater species (*Josephson et al.,* 1984). Later on, *Grimm et al.* (2000) detected this substance in the headspace of cooked catfish and *Girard and Durance* (2000) in the headspace of canned sockeye and pink salmon in traces. The dione could also be identified in fish oil, such as rancid catfish oil and crude menhaden oil (*St. Angelo et al.,* 1987b) and coho salmon oil (*Josephson et al.,* 1991). Moreover, traces of 2,3-octanedione were present in fish sauce (*Peralta et al.,* 1996) and in fish oil enriched mayonnaise (*Hartvigsen et al.,* 2000). Additionally, *Cha et al.* (1992) identified it for the first time in crayfish.

Up to now, the origin of 2,3-octanedione is not fully understood. In many investigations fatty acids or lipids in general are named as possible precursors and the formation of the dione in connection with an oxidative process is reckoned. *Drumm and Spanier* (1991) e.g. admitted that ketones such as 2,3-octanedione are products of lipid oxidation, but that the mechanisms for their formation are not elucidated yet. *Meynier et al.* (1998, 1999) investigated the volatile compounds from oxidised muscle phospholipids of pork and turkey but were not able to identify the structure of the precursor of 2,3-octanedione.

However, a strong correlation between the formation of 2,3-octanedione and of hexanal was observed. This findings led the authors to suggest that the dione and hexanal are a consequence of comparable or similar mechanisms involving n-6 fatty acid oxidation. Studies by *Suzuki* (1985) on the influence of the diet on the flavour of lamb meat led to the suggestion that 2,3-octanedione might be formed as a metabolite from a direct precursor present in grass. Autoxidation of linoleic acid was suggested to be a possible pathway. *Young et al.* (1997) investigated the effect of the diet on the volatiles of lamb fat, and advanced a hypothesis that linked the formation of 2,3-octanedione to the enzyme lipoxygenase and linolenic acid. It is also interesting to note that the formation of 2,3-octanedione in pork during storage was reduced by *Pseudomonas fragi (Chung-Wang et al.*, 1997). *Taylor and Mottram* (1990) identified 2,3-octanedione as an oxidation product of methyl arachidonate, which was confirmed by *Artz et al.* (1993). However, since arachidonic acid is of animal origin, this cannot explain the occurrence of 2,3-octanedione in plants. Recently, *Pompizzi et al.* (2000) identified 2,3-octanedione as a photooxidative degradation product of pentyl DiMeF. Starting from the cycloaddition of singlet oxygen to DiMeF(9,5) to a bicyclic furan endoperoxide, *Pompizzi* (1999) proposed two pathways with either an epoxy dioxoene or a furan diepoxide as an intermediate (Fig. 4).

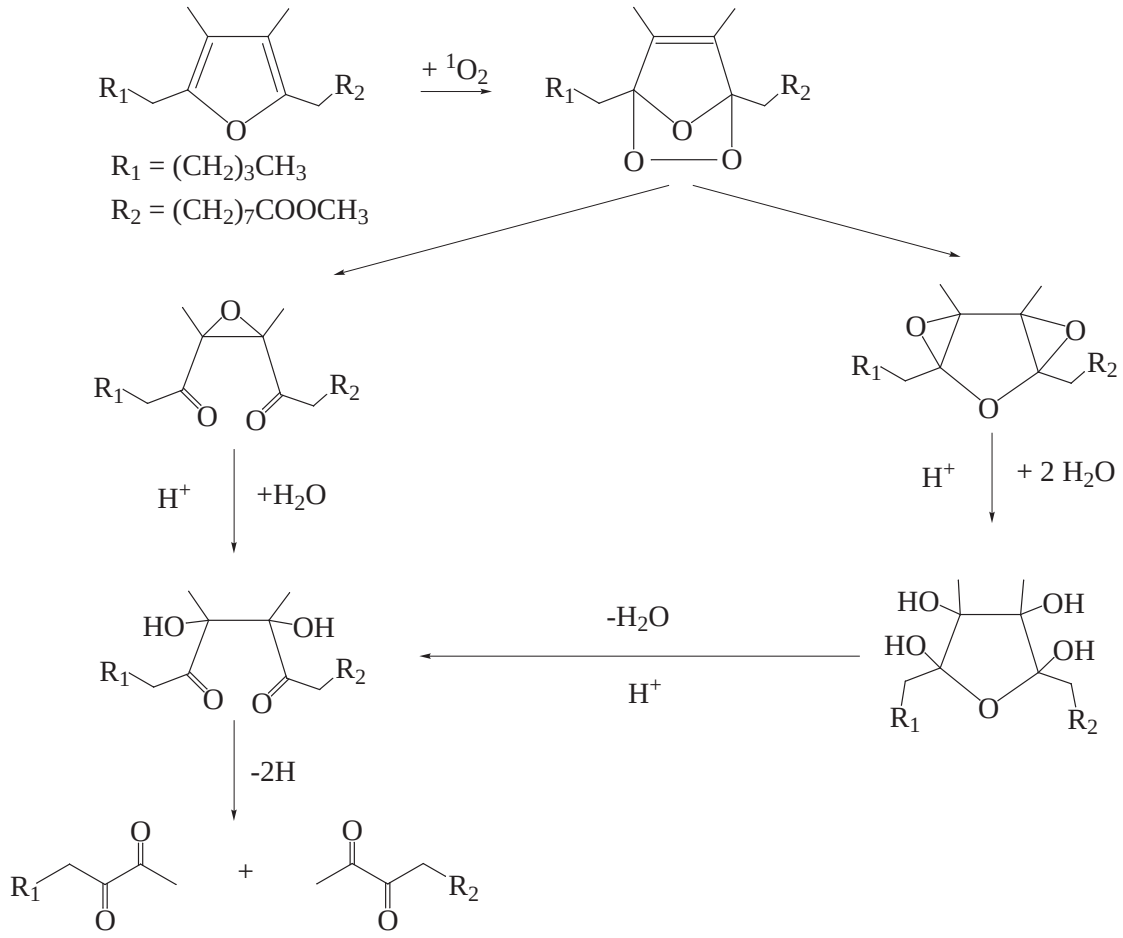


Figure 4: Proposed pathway for the photooxidative formation of 2,3-octanedione (*Pompizzi*, 1999)

Reaction of the epoxide moiety with water leads to the dihydroxy compound which then is oxidised to the corresponding diones. Reaction of the diepoxyde moiety with water leads to the tetra diole, which again gives the dihydroxy compound under elimination of water.

2.2.3 Bovolide and Dihydrobovolide

Bovolide and dihydrobovolide are two of the dimethyl substituted α,β-unsaturated-γ-lactones, which were initially used as artificial flavour additives in tobacco products (*Schumacher and Roberts*, 1966). Some years later, dihydrobovolide (*Kaneko and Mita*, 1969) and bovolide (*Demole and Berthet*, 1972) were isolated from Burley tobacco leaves. These findings were confirmed for example by investigations on the volatile

neutral fraction derived from Burley and Virginia tobacco (*Forsblom et al.*, 1990).

The odour of bovolide is described as celery-like (*Boldingh and Taylor*, 1962; *Mookherjee and Wilson*, 1990), fruity and pleasant (*Takahashi et al.*, 1980), or celery- and lovage-like (*Näf and Velluz*, 2000). Dihydrobovolide possesses a characteristic celery-like aroma as well (*Sakata and Hashizume*, 1973, *Yamanishi et al*, 1973; *Mookherjee and Wilson*, 1990). The odour thresholds of bovolide and of dihydro-bovolide are 100 pg/l and 480 pg/l in air and 2.0 µg/kg and 500 µg/kg in oil orthonasal, respectively (*Pompizzi*, 1999).
Recently, studies by *Kawakami* (2002) revealed a slightly different aroma profile and threshold level of the enantiomers (+)- and (-)-dihydrobovolide. The (+)-enantiomer exhibits a metallic, spicy, green tea note, whereas the (-)-enantiomer develops a light and spicy green tea note. The threshold level of (-)-dihydrobovolide was 1.6 ppm and lower than that of (+)-dihydrobovolide (3.7 ppm).

Bovolide was first identified in butter by *Boldingh and Taylor* (1962) and *Lardelli et al.* (1966) and was named to denote its bovine origin. Later on, the lactone was found in various types of tea, such as green tea (*Horita and Hara*, 1984; 1985), semi fermented Pouchong tea (*Kawakami et al.*, 1986), pickled tea Miang (*Kawakami et al.*, 1987), piled tea Toyama Kurocha (*Kawakami and Shibamoto*, 1991), Oolong tea (*Kawakami et al.*, 1995) and black tea (e.g. *Owuor and Obanda*, 1999). Bovolide has been reported to occur in peppermint oil (*Takahashi et al.*, 1980), soy (*Ames and MacLeod*, 1984), strawberry yam (*Barron and Etiévant*, 1990), essential oil of *Pelargonium* species (*Kayser et al.*, 1998), cooked spinach leaves (*Näf and Velluz*, 2000) and illuminated samples of garden cress, woodruff, wheat germ oil and shrimps (*Pompizzi et al.*, 2000). Additionally, *Bailey et al.* (1994b) detected bovolide in lamb fat.

Dihydrobovolide has been reported to occur in the oil of Japanese peppermint (*Sakata and Hashizume*, 1973), Ceylon flavoured tea (*Yamanishi et al.*, 1973), cooked rice (*Yajima et al.*, 1978), leaves of *Lycium chinense* (*Sannai et al.*, 1983), alfalfa (*Kami*, 1983), blended endive (*Götz-Schmid and Schreier*, 1986), the marine green alga species *Ulva pertusa* (*Fujimura et al.*, 1990), woodruff (*Wörner and Schreier*, 1991) and illuminated dried mushrooms (*Pompizzi et al.*, 2000). In dried bonito, the analogous

compound 2,3-dimethylhept-2-en-4-olide was also identified beside the dihydro-bovolide (*Yajima et al.,* 1983).

Both bovolides have been identified in green tea (e.g. *Horita et al.,* 1985, *Kawakami et al.,* 1989), green mate and roasted mate (*Kawakami and Kobayashi,* 1991), the flower of *Michelia champaca* (*Kaiser,* 1991), Rooibos tea (*Kawakami et al.,* 1993) and dried illuminated lamb‘s lettuce (*Pompizzi et al.,* 2000). In animal samples, bovolide as well as dihydrobovolide were found in cooked beef (*MacLeod,* 1991) and in shrimp powder (*Sarelse et al.,* 1994).

Boldingh and Taylor (1962) assumed that the bovolide found in butterfat originally derived from the fodder. Based on the absence of other homologues they concluded that the biosynthesis of bovolide is not related to normal fatty acid metabolism. *Lardelli et al.* (1966) reported that by chromatographing grass extracts a fraction was obtained with a distinct celery flavour. This was taken as an indication that bovolide is probably present in grass. Further *MacLeod* (1991) assumed that the bovolide in beef originated from grass as fodder. The results of the study by *Bailey et al.* (1994b) on the influence of finishing diets on lamb flavour indicated an origin from grass as well. The authors identified bovolide as one of the compounds having a strong positive relationship with lamby flavour. Fat from animals finished on grain was significantly less lamby in flavour than fat from animals finished on forage.

Horita et al. (1985) found the bovolides to be formed by light-induced chemical reactions. However, in a study on the light-induced off-flavour of green tea, these compounds turned out not to be responsible for the organoleptic sunlight flavour of green tea (*Horita,* 1987).

Sarelse et al. (1994) proposed a pathway for the formation of bovolide by photooxidative degradation of pentyl DiMeF (Fig. 5).

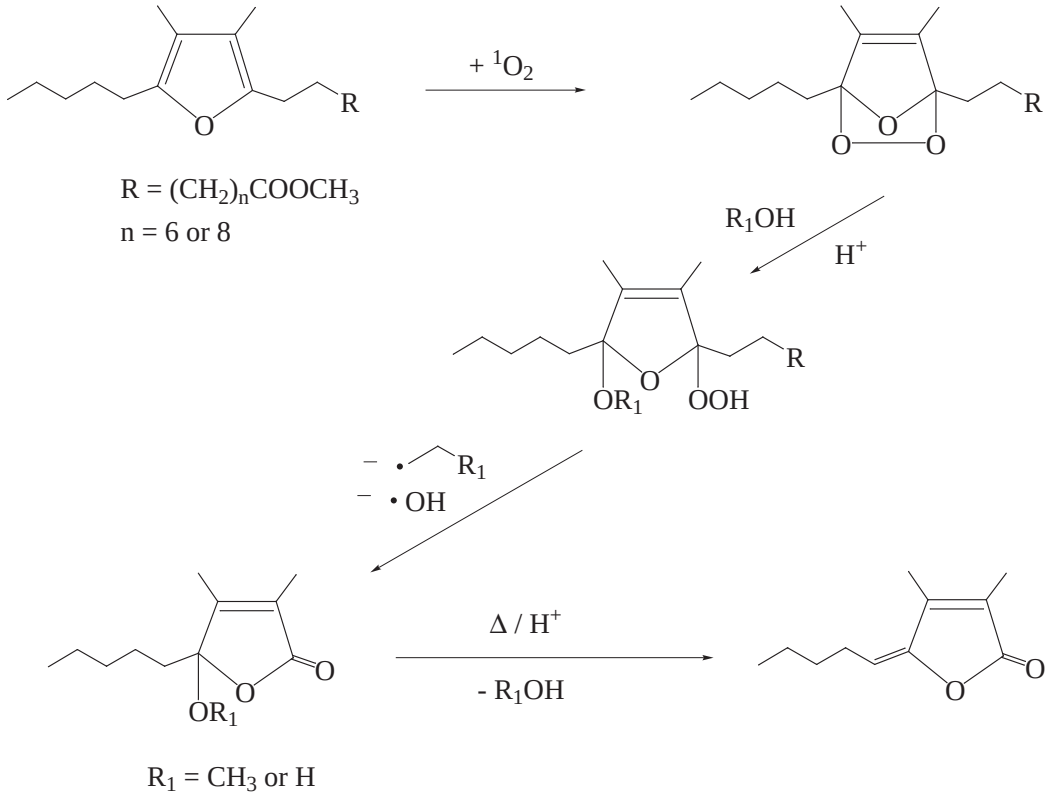


Figure 5: Proposed pathway for the photooxidative formation of bovolide (*Sarelse et al.*, 1994)

Some years later, *Pompizzi et al.* (2000) confirmed the oxidative formation of bovolides from pentyl DiMeF in model experiments and proposed pathways for the autoxidative (Fig. 6) and for the photooxidative formation (Fig. 7) of bovolide. Recently, *Kawakami* (2002) discussed an alternative way for the photooxidative formation of bovolide and dihydrobovolide starting with a fatty acid (Fig. 8).

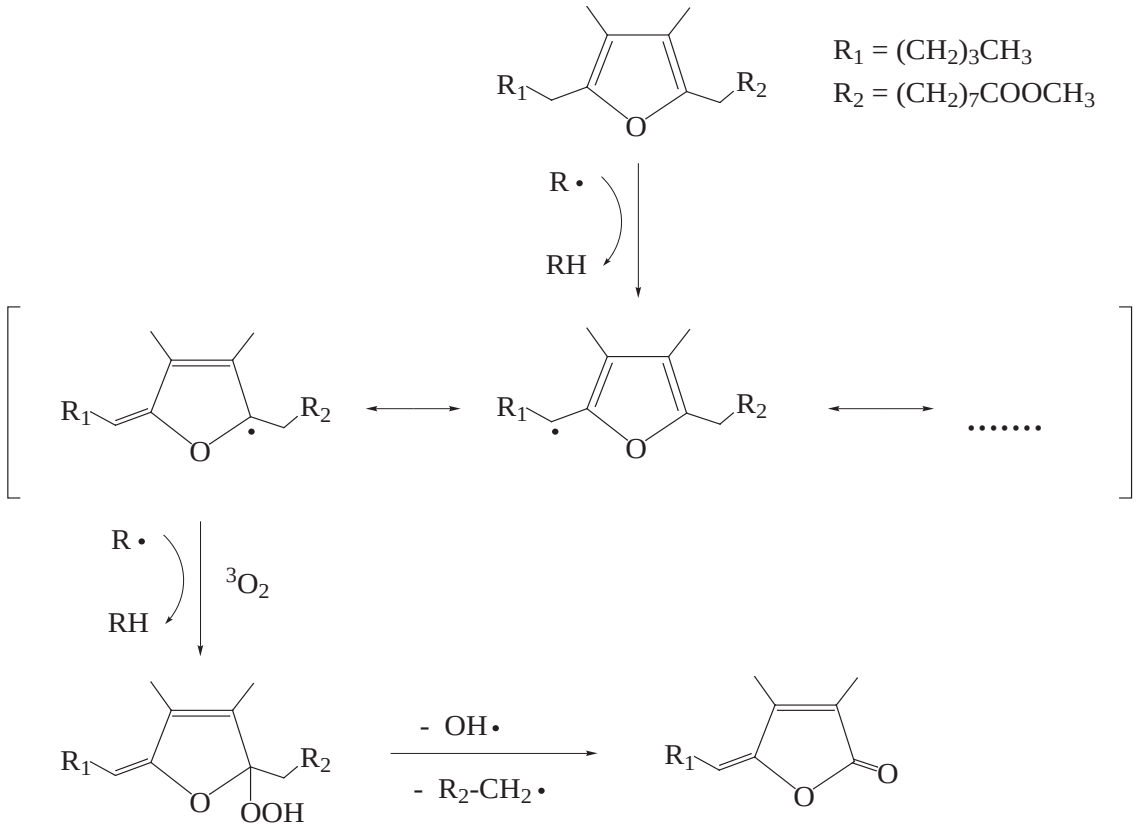


Figure 6: Proposed pathway for the autoxidative formation of bovolide (*Pompizzi et al.*, 1997)

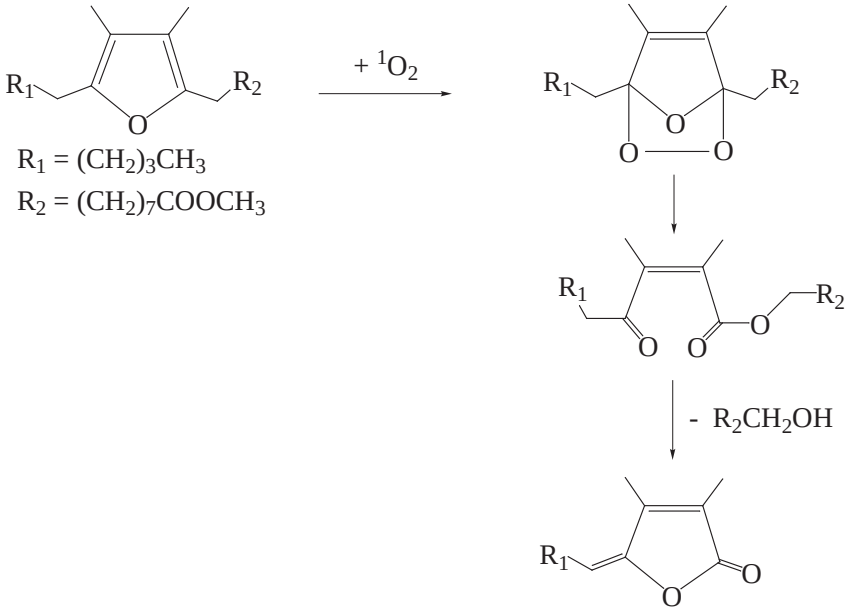


Figure 7: Proposed pathway for the photooxidative formation of bovolide (*Pompizzi et al.*, 2000)

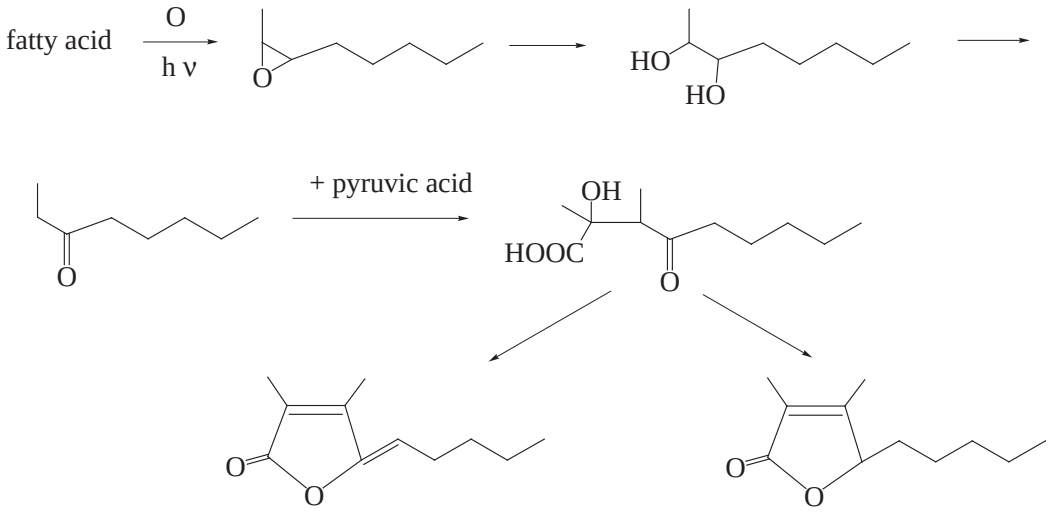


Figure 8: Hypothetical photooxidative formation of bovolide and dihydrobovolide (*Kawakami*, 2002)

2.2.4 Pentanal

According to the general description the odour of pentanal is pungent and almond-like (e.g. *Schnabel et al.*, 1988). In addition, quite different odour perceptions were associated with pentanal such as green, sweet and fatty (*van Ruth et al.*, 1999) and caramel, fruity and musty (*van Ruth et al.*, 2000). Several odour thresholds for pentanal measured in different media have been published (Tab. 4.).

Table 4: Odour thresholds for pentanal in different media

medium	threshold	reference
air	34 ng/l	*Hall and Andersson* (1983)
	39 ng/l	*Boelens and van Gemert* (1987)
water	42 µg/kg (orthonasal)	*Boelens and van Gemert* (1987)
	12 µg/kg (orthonasal)	*Buttery et al.* (1988)
	20 µg/kg (orthonasal)	*Schnabel et al*. (1988)
	76 µg/kg (retronasal)	*Boelens and van Gemert* (1987)
oil	240 µg/kg (orthonasal)	*Boelens and van Gemert* (1987)
	150 µg/kg (retronasal)	*Boelens and van Gemert* (1987)
beef system (meat/water)	267 µg/kg	*Brewer and Vega* (1995)
milk	130 µg/kg	*Boelens and van Gemert* (1987)

Pentanal is a common volatile aroma compound in food. Some published results are worth to be mentioned in connection with the present work. Pentanal has been shown to be the most abundant volatile in field-dried fescue hay (*Mayland et al.*, 1997) and one of the most abundant volatiles in cooked broccoli (*Hansen et al.*, 1997). *Hara* (1989) identified pentanal as one of the photochemically produced off-flavour components of green tea. *Bailey et al.* (1994b) have investigated the influence of finishing diets on lamb

flavour. The fat from animals finished on grain exhibited a significantly less lamby flavour compared to fat from animals finished on forage. Pentanal was part of the volatile compounds which correlated significantly with the lamby flavour strength. *St. Angelo et al.* (1987a) suggested to use pentanal as a marker to follow the development of WOF.

Pentanal is known to be a minor oxidation product of certain fatty acids. This aldehyde was identified as a secondary reaction product of the oxidation of linoleic acid (*Forss,* 1973; *Yoshino et al.,* 1991; *Frankel,* 1998; *Spiteller,* 1998), of linolenic acid (*Yoshino et al.,* 1991), of arachidonic acid (*Forss,* 1973; *Taylor and Mottram,* 1990; *Yoshino et al.,* 1991; *Artz et al.,* 1993), of palmitoleic acid (*Forss,* 1973), of oleic acid (*Neff et al.,* 2000) and of docosahexaenoic acid (*Yoshino et al.,* 1991). *Lardelli et al.* (1966) observed a formation of pentanal by oxidation of bovolide. Recently, *Pompizzi et al.* (2000) identified pentanal as a photooxidative degradation product of pentyl DiMeF.

2.2.5 2,3-Butanedione

2,3-Butanedione (diacetyl) is known as a key aroma compound in butter and, in fact, its generally accepted odour descriptor is buttery (*Widder,* 1994; *Belitz et al.,* 2001). The thresholds have been investigated in many studies in different media and a compilation is given in *Rychlik et al.* (1998). *Siek et al.* (1969) e.g. obtained taste thresholds of 5.4 µg/l in water, 55 µg/l in oil, 14 µg/l in milk and 32 µg/kg in butter.

The dione is a potent aroma compound in milk-products such as yoghurt and cheese, but also in bread, cooked fish, coffee and French fries. In beer and in soy bean-oil, 2,3-butanedione contributes to the off-flavour (*Belitz et al.,* 2001).

Several possible pathways of formation of 2,3-butanedione have been shown. In dairy products, diacetyl is the result of the action of lactic acid bacteria and is biosynthesised from citric acid (*Gatfield,* 1986). *Artz et al.* (1993) identified 2,3-butanedione to be a decomposition product from oxidised methyl arachidonate. *Hollnagel and Kroh* (1998) have shown in model experiments that the dione was a reaction product from non-enzymatic browning of D-glucose, D-fructose, maltose and maltulose. Finally, 2,3-butanedione was identified as a photooxidative degradation product of pentyl DiMeF by *Pompizzi et al.* (2000). However, with the exception of dairy products, the origin of

diacetyl could not be explained for all food products. For example the origin of 2,3-butanedione as potent odorant in boiled trout is still unknown *(Milo and Grosch,* 1993).

3 EXPERIMENTAL PART

3.1 Material

3.1.1 Sample material

Dried samples of tarragon (*Artemisia dracunculus*), basil (*Ocimum basilicum*), savory (*Satureja hortensis*), chervil (*Anthriscus cerefolium*), dill (*Anethum graveolens*), chive (*Allium schoenoprasum*), onion (*Allium cepi*) and leek (*Allium porrum*) were obtained in paper bags from J. Carl Fridlin Gewürze AG, Hünenberg, CH. Green tea samples (Sencha, Fuji region Japan) were obtained in light-protected vacuum-bags from Peter Oppliger AG, Lucerne, CH. The food samples were stored in light-protected vacuum-bags at 4 °C before analysis.

3.1.2 Reference aroma compounds

2,3-Dimethylnona-2,4-dien-4-olide (bovolide) (*Pompizzi*, 1999)
2,3-Butanedione, Fluka 31530 (Fluka AG, Buchs, CH)
2,3-Dimethylnon-2-en-4-olide (dihydrobovolide) (*Pompizzi*, 1999)
3-Methyl-2,4-nonanedione (MND) (see chapter 3.2.1)
2,3-Octanedione (see chapter 3.2.2)
Pentanal, Fluka 94512

3.1.3 Model mixture of reference aroma compounds

A stock solution of the model mixture was prepared by dissolving 20 to 30 mg of each of the aroma compounds pentanal, 2,3-butanedione, 2,3-octandione, MND, bovolide and dihydrobovolide in 20 ml diethyl ether. A diluted aroma solution was prepared by

diluting 1 ml of the stock solution to 10 ml with diethyl ether. In Tab. 5 the exact concentrations of the components of the stock solution are shown.

Table 5: Concentrations of the components of the model mixture in the stock solution

aroma compound	concentration [g/l]
pentanal	1.0
2,3-butanedione	1.5
2,3-octanedione	1.4
MND	0.9
bovolide	1.6
dihydrobovolide	1.1

3.1.4 Dimethyl furan fatty acid standards

DiMeF(7,5)methyl ester, DiMeF(8,5)methyl ester, DiMeF(9,5)methyl ester, DiMeF(11,5)methyl ester, DiMeF(11,3)methyl ester (*Pompizzi*, 1999).

3.1.5 Chemicals

All chemicals were purchased from J.T. Baker B.V. (Deventer, NL), Fluka AG (Buchs, CH), Lancaster Synthesis GmbH (Frankfurt a. Main, D), Merck Ltd. (Darmstadt, D), Riedel-de Haën Laborchemikalien GmbH & Co KG (Seelze, D), Separtis GmbH (Grenzach-Wyhlen, D) or Siegfried AG (Synopharm, Basel, CH) and were of analytical quality.

Acetonitrile (Fluka 00700), boron trifluoride-methanol 10 % (Fluka 15716), carbon tetrachloride (Fluka 87031), copper(II)acetate (Riedel-de Haën 25038), dichloro-methane (Baker 7053), diethyl ether (Riedel-de Haën 32203), 2,6-di-tert-butyl-p-hydroxy-toluene (BHT) (Fluka 34750), ethanol (Fluka 02860), ethyl decanoate (Fluka 21430), ethyl valerate (Fluka 94540), hexane (Fluka 52760), iodomethane

(Fluka 67690), isolute HM-N (Separtis 9800-0060), isopropanol (Riedel-de Haën 27225), magnesium sulfate anhydrous (Fluka 63136), meso-tetraphenyl porphyrine (Fluka 88071), methanol absolute (Fluka 65543), methanol (Baker 8045), methylene blue (Fluka 66720), methyl undecanoate (Fluka 94118), 2,4-nonanedione (Lancaster 1151), 2-octine (Fluka 74972), potassium hydroxide (Siegfried 16200-06), ruthenium(IV)oxide (Fluka 84065), silica gel 60 (Fluka 60752), sodium (Fluka 71172), sodium chloride (Merck 6400), sodium hydrogen carbonate (Fluka 71628), sodium periodate (Fluka 71862), sulfuric acid (Merck 100731).

3.2 Synthesis of reference aroma compounds

3.2.1 3-Methyl-2,4-nonanedione

MND was synthesised according to *Guth and Grosch* (1989). A solution of sodium ethylate was prepared by dropwise addition of 9 ml ethanol to 0.4 g (16 mmol) sodium under stirring. After complete dissolution of the sodium, the solution was heated to 70 °C and treated dropwise under stirring with 2.8 g (16 mmol) 2,4-nonanedione and 1.1 ml (16 mmol) iodomethane. The mixture was refluxed at 75 °C for 4 h, treated with additional 0.5 ml (8 mmol) iodomethane and refluxed at 80 °C for 1 h. Most of the ethanol was then distilled off and the residue poured in 100 ml ice water. After extraction of the mixture with diethyl ether (3 x 150 ml), the combined ethereal extracts were washed with brine, dried ($MgSO_4$) and concentrated under reduced pressure. The yield of crude MND, obtained as a yellow oil, was 3.2 g (102 %) with a purity of 81 % (GC-FID).

The purification was performed according to *Adams and Hauser* (1944). To 0.6 g of crude MND, 30 ml of a hot 7 % (w/v) copper(II)acetate solution was added and the mixture refluxed under stirring at 80 °C for 10 min. After cooling to 0 °C, the mixture was filtered, the residue (copper salt) washed with ice-cold methanol (2 x 10 ml) and recrystallised from boiling methanol. The whitish crystals were dissolved in 10 ml of a 10 % (w/v) sulfuric acid solution and the solution extracted with diethyl ether (3 x 20 ml). The combined ether extracts were washed with brine to neutral, dried ($MgSO_4$) and concentrated under reduced pressure. The yield of MND, obtained as pale yellow oil, was 0.29 g (48 %) with a purity of 98 % (GC-FID). The analytical data were in agreement with those reported by *Pompizzi* (1999).

3.2.2 2,3-Octanedione

According to *Zibuck and Seebach* (1988), 39.8 g (186.1 mmol) sodium periodate was added to a mixture of 5 g (45.5 mmol) 2-octine in 280 ml water, 200 ml acetonitrile and 200 ml carbon tetrachloride. The mixture was stirred vigorously at room temperature until two clear phases resulted. Then 133 mg (0.8 mmol) ruthenium(IV)oxide was added under ice cooling. The mixture was stirred at room temperature for 30 min. After addition of 300 ml water, the organic phase was separated, the aqueous phase extracted with dichloromethane (3 x 100 ml), the combined organic phase dried ($MgSO_4$) and concentrated by means of a Vigreux column. The residue (2.19 g, 33.9 %) was purified by chromatography (silica gel 60, l = 40 cm, d = 7 cm, hexane/dichloromethane = 1:1 (v/v)), distilled at reduced pressure in a Kugelrohr to yield 1.55 g (70 %) 2,3-octanedione as pale yellow oil with a purity of 97 % (GC-FID). The analytical data were in agreement with those reported by *Pompizzi* (1999).

3.3 Analytical methods

3.3.1 Capillary gas chromatography (GC-FID)

GC was performed using the on column (OC) injection technique on a Trace GC 2000 series gas chromatograph (ThermoQuest CE Instruments Ltd., Milano, I) with flame ionisation detector (FID). A polar fused silica SW-10 column (60 m, 320 µm ID, 0.25 µm film thickness, Supelco Inc., Bellefonte, USA) with a deactivated fused silica pre-column (2.8 m, 530 µm ID, J & W Scientific Inc., Folsom, USA) was used. Data processing was achieved with ChromCard, version 1.06 software (ThermoQuest CE Instruments). Tab. 6 shows the experimental conditions for GC analysis. For semi-quantitative analysis, the peak area ratio (R_{FID}) to an internal standard (ISTD) was determined according to equation 1.

Table 6: Experimental conditions for GC-FID analysis

carrier gas	He
carrier flow [ml/min]	1.8
injection mode	OC
injection temperature [°C]	20
injection volume [µl]	13
detection temperature [°C]	240
temperature programming	
isothermal stage 1 [°C]; [min]	40; 18
rate 1 [°C/min]	6
isothermal stage 2 [°C]; [min]	220; 15

$$R_{FID} = \frac{A_X}{A_{ISTD}} \qquad \text{(equation 1)}$$

where: R_{FID}: relative amount of compound X as compared to the internal standard
A_X: peak area of compound X
A_{ISTD}: peak area of internal standard

3.3.2 Capillary gas chromatography-mass spectrometry (GC-MS)

GC-MS was performed using the OC injection technique on a GC 8065 gas chromatograph (Fisons Instruments Inc., Milano, I) directly coupled to a SSQ 710 mass spectrometer (Finnigan Inc., San Jose, USA). A polar fused silica SW-10 column (60 m, 320 µm ID, 0.25 µm film thickness, Supelco) with a deactivated fused silica pre-column

(2.8 m, 530 µm ID, J & W Scientific) was used. Data processing was achieved with the ICIS 2 version 8 software (Finnigan). Tab. 7 shows the three different sets of conditions for GC-MS analysis. Semi-quantitative analysis was performed by evaluation of the peak area ratio (R_{MS}) to an internal standard (see also equation 1) based either on the total ion current (TIC) or the characteristic fragment ions at m/z 58 (pentanal), 74 (methyl undecanoate), 83 (dihydrobovolide), 86 (2,3-butanedione), 88 (3-hydroxy-3-methyl-2,4-nonanedione (HMND), ethyl decanoate, ethyl valerate), 99 (2,3-octanedione, MND) and 124 (bovolide).

.

Table 7: Experimental conditions for GC-MS analysis

method	A	B	C
carrier gas	He	He	He
carrier flow [kPa]	100	100	100
injection mode	OC	OC	OC
injection temperature [°C]	20	20	20
injection volume [µl]	10	1	1
temperature programming			
isothermal stage 1 [°C]; [min]	40; 12	50; 5	90; 5
rate 1 [°C/min]	6	10	10
isothermal stage 2 [°C]; [min]	220; 30	240; 10	240; 10
ionisation potential [eV]	70	70	70
ion source temperature [°C]	150	150	150
manifold temperature [°C]	70	70	70
interface temperature [°C]	200	200	200
mass range [amu]	40-420	40-420	40-420
scan time [s]	1	1	1

3.3.3 Capillary gas chromatography-ion trap mass spectrometry (GC-MS/MS)

GC-MS/MS was performed either on a Trace GC 2000 series gas chromatograph (ThermoQuest CE Instruments) directly coupled to a GCQ Plus mass spectrometer (ThermoQuest Finnigan Inc, San Jose, USA) using an apolar fused silica column Rtx-5MS (30 m, 250 µm ID, 0.25 µm film thickness, Restek Corp., Bellefonte, USA) or on a Varian 3800 gas chromatograph (Varian Inc., Palo Alto, USA) directly coupled to a Varian Saturn 2000 mass spectrometer (Varian) using an apolar fused silica column DB5-MS (28 m, 250 µm ID, 0.25 µm film thickness, J & W Scientific). The experimental conditions for the GC-MS/MS analysis and the MS/MS ion preparation are listed in Tab. 8 and Tab. 9.

Table 8: Experimental conditions for GC-MS/MS analysis

method	D	E
facility	ThermoQuest	Varian
carrier gas	He	He
carrier flow [ml/min]	1.6	1.6
injection mode	splitless 1.0 min	splitless 0.5 min
injection temperature [°C]	250	250
injection volume [µl]	1	2
temperature programming		
isothermal stage 1 [°C]; [min]	60; 1	60; 1
rate 1 [°C/min]	10	10
isothermal stage 2 [°C]; [min]	300;10	300;10
ionisation potential [eV]	70	70
ion source temperature [°C]	150	150
manifold temperature [°C]	70	70
interface temperature [°C]	275	250

Data processing was achieved with the XCalibur version 1.1 software (ThermoQuest Finnigan) or with the Saturn View (TM) version 5.41 software (Varian), respectively. Samples were injected in the splitless injection mode.
For quantification, a six or eight point calibration curve (see chapter 4.1.1) was established and for semi-quantitative analysis, the peak area ratio ($R_{MS/MS}$) to an internal standard was determined (see also equation 1). For both calculations the characteristic product ions at m/z 123 (pentyl DiMeF) and 109 (propyl DiMeF) were used.

Table 9: Experimental conditions for the MS/MS ion preparation

method	D	E
isolation parameters:		
precursor ion [amu]	179 (pentyl DiMeF) 151 (propyl DiMeF)	179 (pentyl DiMeF) 151 (propyl DiMeF)
range [amu]	1	1
isolation time [ms]	12	10
dissociation parameters:		
collision energy [V]	1.2	1.2
collision time [ms]	15	12
q_z value	0.45	0.45

3.3.4 Capillary gas chromatography-infrared spectrometry (GC-IR)

GC-IR was performed using the OC injection technique on a HP 5890 gas chromatograph (Hewlett Packard Co, Palo Alto, USA) with a HP 5965 IR-detector (Hewlett Packard). A medium-polar fused silica DB 1701 column (10 m, 180 µm ID, 0.4 µm film thickness, J & W Scientific) was used. Data processing was achieved with the GRAMS/32 software (Portmann Instruments AG, Biel-Benken, CH). Tab. 10 shows the experimental conditions for GC-IR analysis.

Table 10: Experimental conditions for GC-IR analysis

carrier gas	He
carrier flow [kPa]	100
injection mode	OC
injection temperature [°C]	20
injection volume [µl]	2
temperature programming	
isothermal stage 1 [°C]; [min]	40; 1
rate 1 [°C/min]	10
isothermal stage 2 [°C]; [min]	300; 10

3.3.5 Capillary gas chromatography-olfactometry (GC-O)

For sniffing experiments, a GC-FID system of the type HP GC 5890 series II (Hewlett Packard) was equipped with a column end split, leading to a sniffing port for olfactometry. A polar fused silica SW-10 column (60 m, 320 µm ID, 0.25 µm film thickness, Supelco) was used. Data processing was achieved with a HP 3365 Series II integrator (Hewlett Packard) and the Chem Station Version A.03.34 software (Hewlett Packard). Samples were injected in the split injection mode. Tab. 11 shows the experimental conditions for GC-O analysis.

Table 11: Experimental conditions for GC-O analysis

carrier gas	He
carrier flow [kPa]	100
injection mode	split 1:12
injection temperature [°C]	240
injection volume [µl]	2
detection temperature [°C]	240
temperature programming	
isothermal stage 1 [°C]; [min]	90; 7
rate 1 [°C/min]	20
isothermal stage 2 [°C]; [min]	150; 10
rate 2 [°C/min]	20
isothermal stage 3 [°C]; [min]	240; 10

3.4 Extraction methods

3.4.1 Simultaneous distillation solvent extraction (SDE)

The extraction of the volatile compounds was performed in a micro steam distillation apparatus (Chrompack Inc., Middelburg, NL) modified for solvents lighter than water according to the design of *Godefroot et al*. (1981). 170 ml water, 200 µl of the diluted aroma solution (see chapter 3.1.3) or 10.0 g of sample material and 21 µg ethyl decanoate as internal standard (ISTD 1) were placed in a 250 ml roundbottom flask. For herbs and vegetables 150 ml water, 6.0 g sample material and 12 µg ISTD 1 were used. In a 5 ml pear-shaped flask 2 ml diethyl ether and 20 µg ethyl valerate as internal standard (ISTD 2) were placed. The aqueous mixture was heated to boiling and the organic solvent containing flask was heated in an oil bath set at 80 °C. The condenser was cooled to -17 °C and micro-SDE carried out for 60 min after the water vapour reached the condenser. After removing the heating sources, the solvent in the separation chamber was transferred to the extract, 21 µg methyl undecanoate as internal standard

(ISTD 3) were added and the extract dried ($MgSO_4$). The samples were analysed with GC-FID (Tab. 6) and/or GC-MS (Tab. 7, method A). The compounds were identified by comparison of mass spectra and retention indices (RI, calculated according to *Van den Dool and Kratz* (1962)) with reference substances. R_{FID} and R_{MS} were calculated with either ISTD 1 or ISTD 3 as internal standards. To investigate a possible artefact formation, a solution of 110 µg DiMeF(7,5)methyl ester, 110 µg DiMeF(9,5)methyl ester and 160 µg DiMeF(11,5)methyl ester dissolved in 100 µl diethyl ether was subjected to the same procedure.

3.4.2 Accelerated solvent extraction (ASE)

For the analysis of the FFA, the lipid fraction was extracted by accelerated solvent extraction (ASE) using an ASE 200 equipment (Dionex Corp. Sunnyvale, USA). The samples were finely ground immediately before extraction. To prevent oxidation during the extraction, the solvent was degassed before use and 0.1 % BHT (w/v) was added. The extraction conditions are listed in Tab. 12. The extract was dried ($MgSO_4$) and concentrated at reduced pressure.

The crude lipids were saponified under argon with 25 ml 0,5 M potassium hydroxide in methanol for 15 min at reflux and then converted to methyl esters by refluxing under argon with 20 ml 10 % (1.3 M) boron trifluoride in methanol for 5 min. After addition of 100 ml satd. sodium hydrogen carbonate solution, 40 ml hexane and 280 µg DiMeF(8,5)methyl ester as internal standard, the mixture was stirred for 10 min at room temperature and extracted with hexane (5 x 40 ml). The organic phase was washed with brine (2 x 40 ml), dried ($MgSO_4$) and concentrated at reduced pressure. The residue was stored under argon at -28 °C before analysis. For the GC-MS/MS analysis (for quantification: method D (Tab. 8 and 9); for semi-quantitative analysis: method E (Tab. 8 and 9)), aliquots were dissolved in hexane. The compounds were identified by comparison of mass spectra, characteristic product ions and retention times with DiMeF standards. Quantification and calculation of $R_{MS/MS}$ were performed with DiMeF(8,5)methyl ester as internal standard.

Table 12: Operating parameter for the ASE 200 extraction

sample amount [g]	approx. 10
hydromatrix [g]	approx. 2 (isolute HM-N)
pressure [MPa]	6,7
temperature [°C]	125
solvent [v/v]	hexane-isopropanol (3:2)
heating up phase [min]	6
static time [min]	10
static cycles	2
flush volume [% of cell volume]	60
nitrogen flush [min]	3
extraction steps per sample	4

3.5 Light exposure experiments

3.5.1 Sample preparation

The dried samples were packed under air or oxygen atmosphere in transparent PE-film bags and exposed to light (see chapter 3.5.2 and chapter 3.5.3) at room temperature for different time periods. Samples before light exposure or stored in darkness under the same conditions were used as reference.

3.5.2 Light exposure model system I

The experiments were carried out in a cardboard box (length = 115 cm, width = 47 cm, height = 90 cm) with two fluorescent tubes (BIOLUX 36W/72-965, Osram AG, Winterthur, CH) with an emission spectrum similar to that of the sun (Fig. 9). The samples were placed at a distance of 45 cm from the light source. The illuminance was adjustable from 1100 lx to 4500 lx. A regulation system with a photo cell (*Reiter*, 2000)

ensured a constant illuminance during the exposure period.

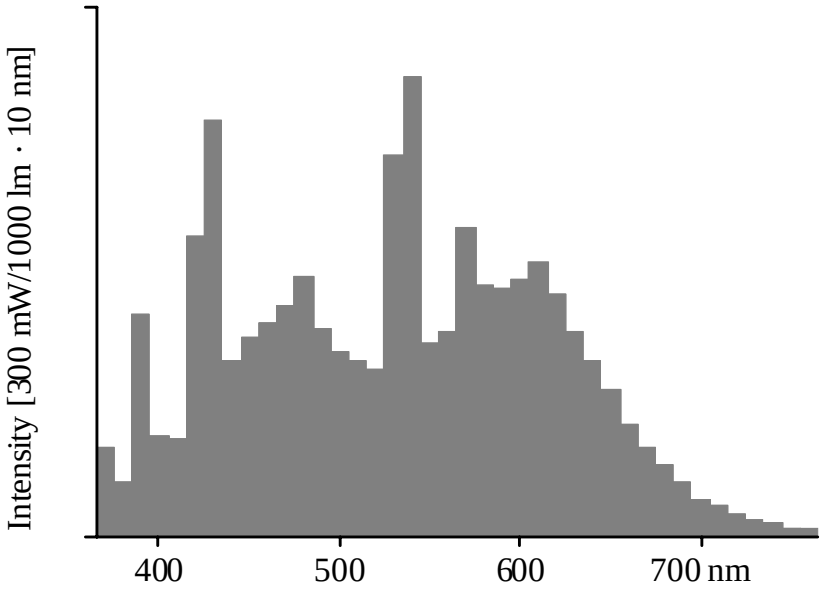


Figure 9: Emission spectrum of the BIOLUX lamps (*Osram*, 1995/1996a)

3.5.3 Light exposure model system II

The experiments were carried out in a dark room. The light source consisted of two sodium vapour-high pressure lamps (Vialox NAV E 140 de Luxe, Osram) placed at a distance of 30 cm from the samples. The illuminance corresponded to 40‘000 lx. The emission spectrum of the lamps was similar to that of the sun (Fig. 10).

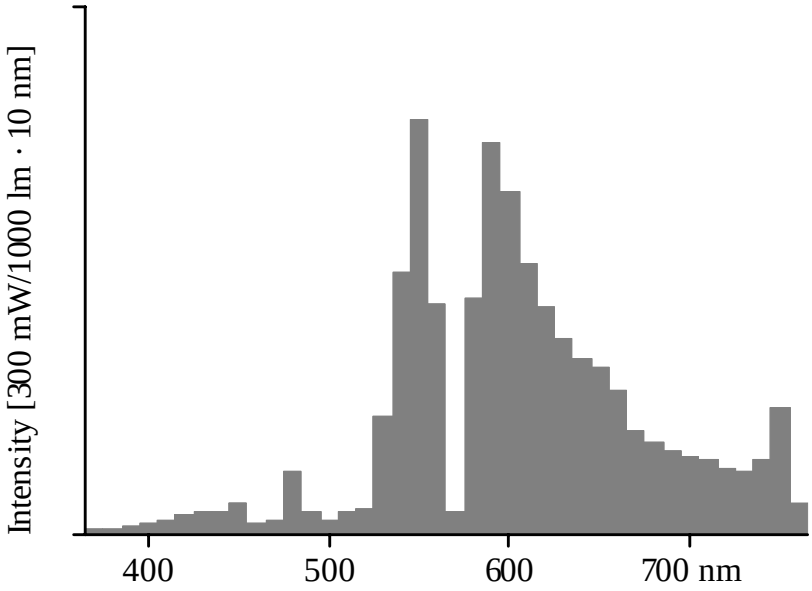


Figure 10: Emission spectrum of the Vialox lamps (*Osram*, 1995/1996b)

3.6 Oxidation experiments

The experiments were performed under photooxidative conditions at 4 °C in two different organic solvents according to *Pompizzi* (1999). A special three neck glass vessel (d_1 = 4 cm, d_2 = 10 cm, h = 14 cm) with a fritted glass bottom (DEMA 13/21, Hans Mangels, Bornheim, D) was used. The light source consisted of two sodium vapour-high pressure lamps (Vialox NAV E 150 de Luxe, Osram) with an illuminance of 20‘000 lx each placed at a distance of 0.45 m from the reaction vessel.

Experiments with MND: 34 µg (0.17 mmol) MND, 144 µg ethyl decanoate (internal standard) and 69 µg ethyl valerate (internal standard).
Experiments with 2,3-octanedione: 28 µg (0.2 mmol) 2,3-octanedione, 151 µg ethyl decanoate (internal standard) and 52 µg ethyl valerate (internal standard).

The oxidation products were identified by comparison of RI and of the mass spectra by spiking with reference substances. If reference substances were not available, identification was based on mass spectral characteristics. The main degradation products were analysed semi-quantitatively (R_{MS} based on TIC) with the internal standard ethyl decanoate.

3.6.1 Conditions in hexane

In 200 ml hexane, 20 mg meso-tetraphenyl porphyrine was dissolved with the aid of an ultrasonic bath and the dione and the two internal standards were added. The solution was illuminated for 24 h under slightly bubbling oxygen. After 24 h, 0.6 mg BHT were added, and the solution was concentrated to 3 ml at reduced pressure (50 - 60 mbar) by means of a Vigreux column and ice cooling.
The same procedure was carried out in absence of light (dark oxidation), absence of sensibilisator (unsensitised oxidation) and in absence of sensibilisator and argon instead of oxygen (photochemical reaction).
The samples were stored at -80 °C before GC-MS analysis with method C (Tab. 7).

3.6.2 Conditions in methanol

A solution of the dione, the two internal standards and 30 mg methylene blue in 200 ml methanol was illuminated for 24 h under slightly bubbling oxygen. After 24 h, 0.6 mg BHT were added, and the solution was concentrated to 3 ml at reduced pressure (35 mbar) by means of a Vigreux column and ice cooling. The residue was diluted with 10 ml water and the mixture extracted with diethyl ether (3 x 10 ml). The combined organic phase was dried ($MgSO_4$) and concentrated to 3 ml at 120 mbar by means of a Vigreux column and ice cooling.
The same procedure was carried out in absence of light (dark oxidation). The samples were stored at -80 °C before GC-MS analysis with method B (Tab. 7).

4 RESULTS AND DISCUSSION

4.1 Analysis of furan fatty acids

4.1.1 Development of a method using ion trap gas chromatography-mass spectrometry

In order to develop an appropriate GC-MS/MS method for the analysis of FFA two points had to be clarified. At first, the main diagnostic ion of each class of FFA (pentyl DiMeF and propyl DiMeF) was determined. Secondly, the conditions to generate these diagnostic ions in preferably high intensity were optimised. The objective was to attain a satisfactory sensitivity even in complex samples.

DiMeF show analogous fragmentation with allylic cleavage of the carboxyl side chains (*Wahl*, 1994). The resulting base peak is therefore only dependent on the alkyl side chain with m/z 179 for the pentyl DiMeF and m/z 151 for the propyl DiMeF. These characteristic fragment ions were chosen for further fragmentation. MS/MS experiments of the precursor ion at m/z 179 resulted in a characteristic base peak at m/z 123 by allylic cleavage of the pentyl side chain (Fig. 11 A). The peak at m/z 123 was chosen as diagnostic ion for the analysis of pentyl DiMeF. Fragmentation of the precursor ion for propyl DiMeF at m/z 151 surprisingly led to a characteristic base peak at m/z 109 (Fig. 11 B). It is assumed that a vinylic cleavage of the propyl side chain dominated over the allylic cleavage at the MS/MS conditions used in this study. The peak at m/z 109 was therefore chosen as diagnostic ion for the analysis of propyl DiMeF.

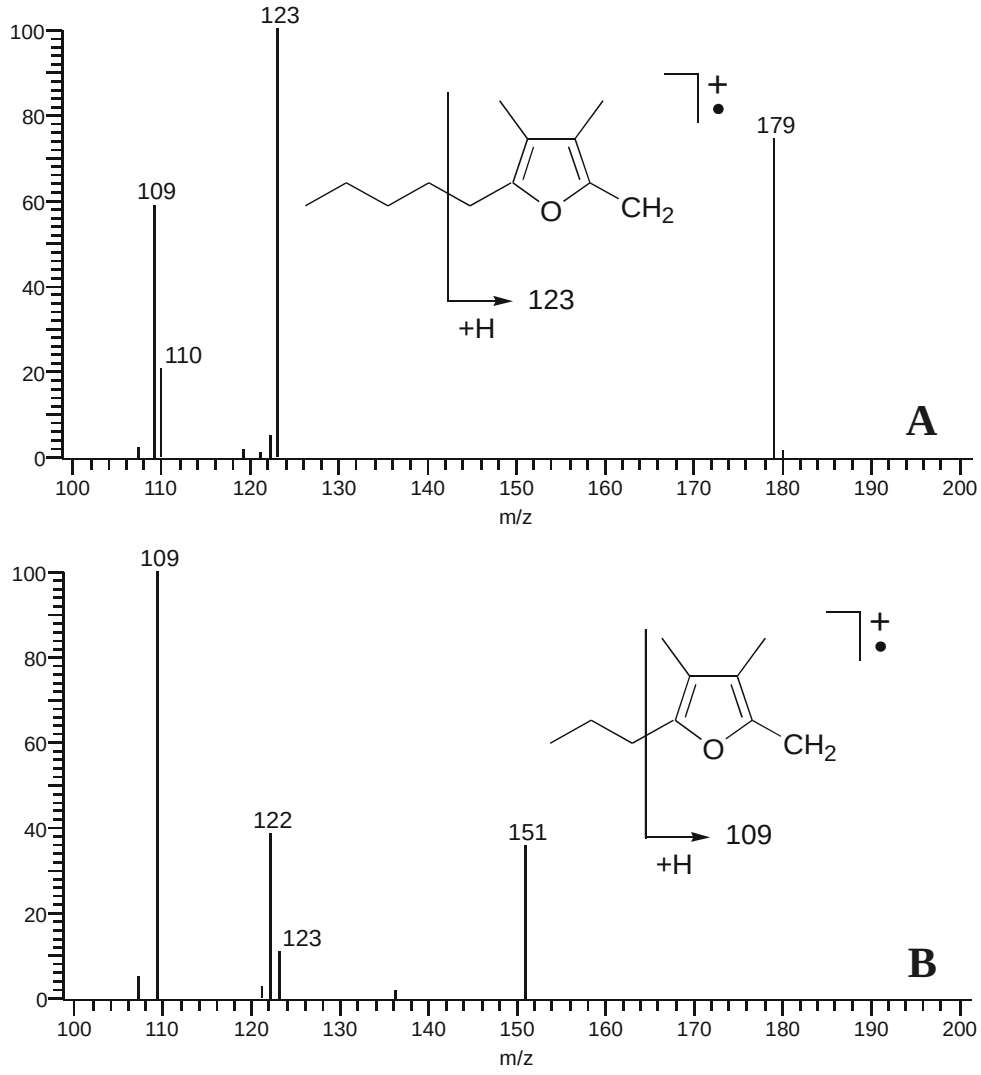


Figure 11: MS/MS spectrum of characteristic precursor ions for pentyl and propyl dimethyl furan fatty acids
A: m/z 179
B: m/z 151

As an example for the efficiency of the method, the ion trap GC-MS chromatograms of the fatty acid methyl ester extract of chervil are shown in Fig. 12. In the GC-MS analysis (Fig. 12 A), the FFA either co-eluted with other components present in the extract or could not be detected. With GC-MS/MS (Fig. 12 B), different pentyl DiMeF were separated in the product ion trace of the diagnostic ion at m/z 123.

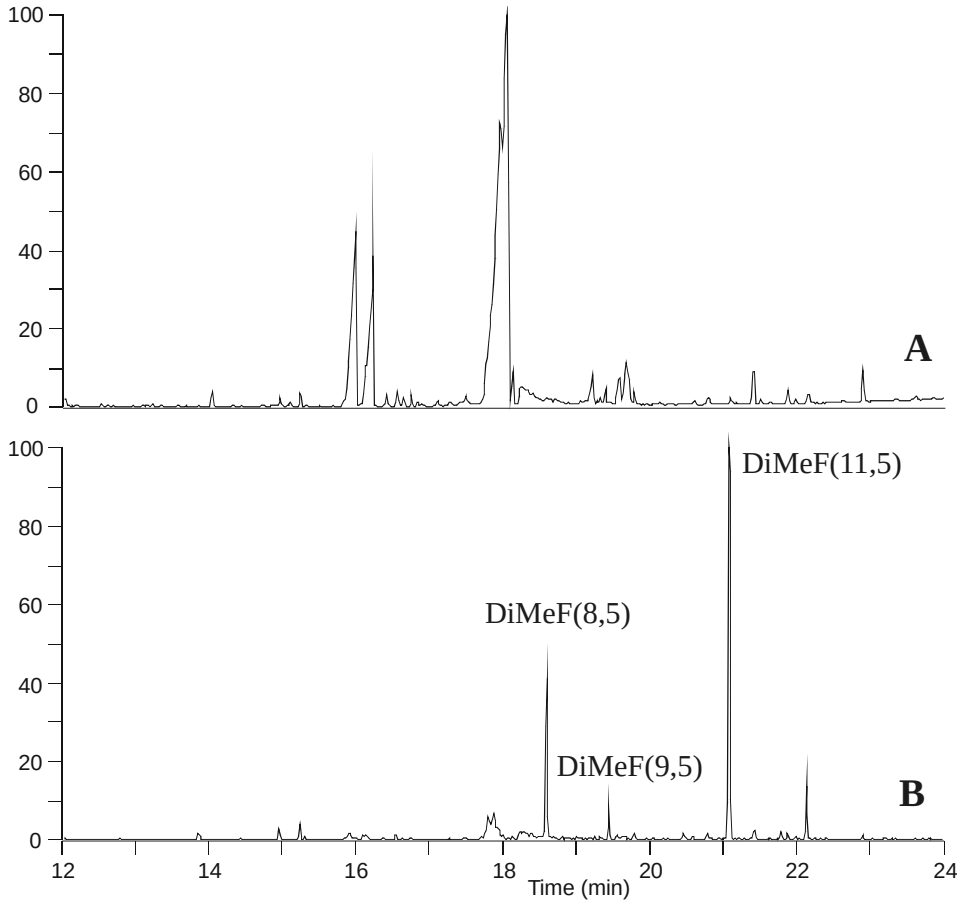


Figure 12: Ion trap GC-MS chromatograms of fatty acid methyl ester extract of chervil
(A) MS chromatogram, based on full scan (m/z 40-450)
(B) MS/MS chromatogram, based on the diagnostic product ion (m/z 123)

Grabic et al. (2000) stated that at a constant isolation time and excitation time the yield of product ions from an MS/MS experiment depends on the collision energy added to the precursor ions. The evolution of intensity of the product ion as a function of the excitation voltage was tested at a constant q_z value = 0.45, isolation time of 12 ms and excitation time of 15 ms. The analysis was performed in two steps. Firstly the peak area of the chosen diagnostic ion was determined with excitation voltages between 0 and 2 V. Secondly the excitation voltage was varied in the area of the higher intensity. In addition to a high peak area of the product ion, incomplete fragmentation of the precursor ion was a matter of concern as well. The relationship between collision energy and yield of the diagnostic ions for pentyl DiMeF and propyl DiMeF was established with DiMeF(9,5) and DiMeF(11,3) standards, respectively (Tab. 13 and Tab. 14).

Table 13: Intensity of the diagnostic ion for pentyl DiMeF at varying collision energy

excitation voltage [V]	peak area diagnostic ion (m/z 123)	precursor ion available (m/z 179)
0	-	yes
1.0	10‘775‘745	yes
2.0	2‘763‘032	no
0.9	10‘371‘469	yes
1.2	11‘853‘346	yes
1.5	4‘729‘902	yes

Table 14: Intensity of the diagnostic ion for propyl DiMeF at varying collision energy

excitation voltage [V]	peak area diagnostic ion (m/z 109)	precursor ion available (m/z 151)
0	-	yes
0.9	40‘788‘468	yes
1.2	50‘608‘840	yes
1.5	13‘874‘301	no
0.9	52‘525‘853	yes
1.0	54‘365‘911	yes
1.2	55‘019‘434	yes

The highest intensity of the target product ions was obtained at an excitation voltage of 1.2 V for both the pentyl DiMeF and the propyl DiMeF. At low excitation voltages the fragmentation of the precursor ions was too low. On the other hand, at high excitation voltages too many low-molecular weight product ions were formed, which were not hold back in the ion trap at the chosen q_z value. Duplication of the excitation time to 30 ms did not enhance the intensity of the diagnostic ions (data not shown). Therefore a possible effect of the excitation time as well as the isolation time was supposed to be negligible and was not further studied. *Grabic et al.* (2000) have not observed a significant improvement in sensitivity at different values of excitation time and isolation time in the analysis of PCDDs and PCDFs. However, the authors obtained a higher response of the product ion at an increased collision pressure compared to a lower one. It is likely that the sensitivity in the analysis of FFA could be enhanced as well by optimising the pressure of the collision gas.

Based on the expected allylic cleavage of the alkyl and carboxyl side chains (Fig. 13), the precursor ions and diagnostic product ions of different classes of FFA were deduced. This allowed a qualitative screening of homologous mono- and dimethyl substituted FFA in the samples without using standards for verification. As discussed for propyl DiMeF, vinylic cleavage of the alkyl side chain can dominate the allylic cleavage. In this case, qualitative analysis using the predicted diagnostic ion is still possible, however, with a decreased sensitivity. For identification of the FFA, the corresponding molecular ion was taken as an additional criterion. The second fragmentation of some components present in the methyl ester extracts, however, led to ions with m/z values identical to the suggested diagnostic product ions of the FFA. In these cases, the complete MS/MS fragmentation spectra were interpreted to differentiate peaks not derived from FFA.

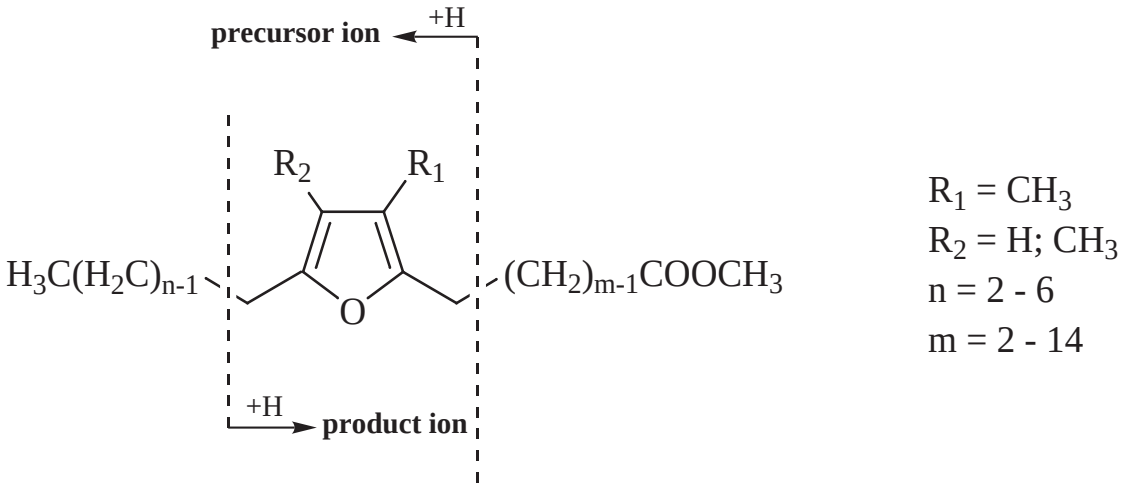


Figure 13: Precursor ion and product ion obtained by allylic cleavage of furan fatty acid methyl esters

In the samples, DiMeF(9,5) and DiMeF(11,5) as well as DiMeF(11,3) were quantified with the internal standard method. DiMeF(8,5) was used as ISTD since DiMeF with an odd number of carbon atoms have not been reported to occur in plants yet. Because of the wide range of concentrations of DiMeF(11,5) in the different samples ranging from 0.5 mg/kg to over 300 mg/kg, two separate calibration curves were established. For the other two FFA, calibration curves were established in the range of the expected concentrations in the samples. Mean values of independent duplicates were used to calculate the calibration graphs (Fig. 14).

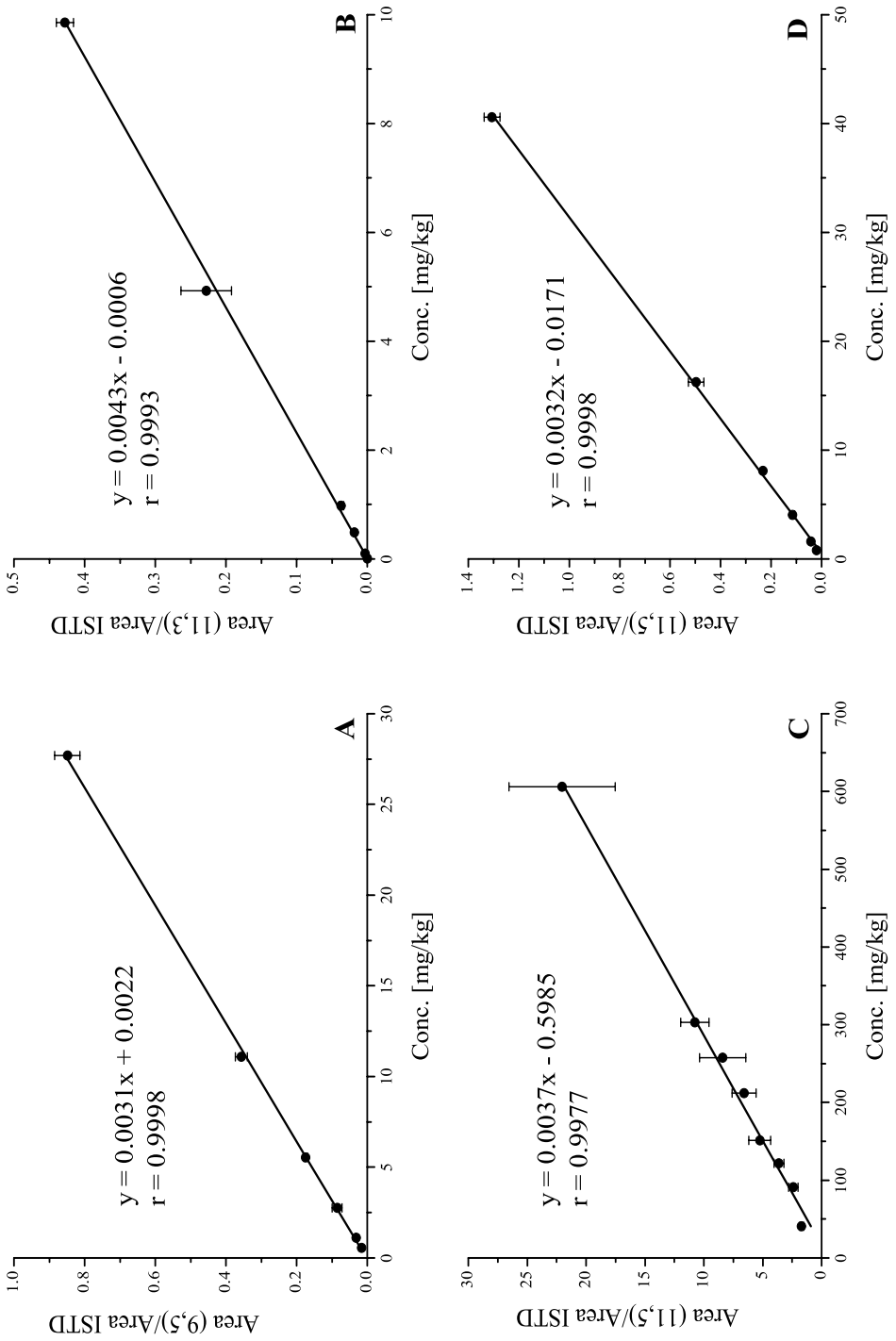


Figure 14: Calibration graphs for quantification of different furan fatty acids (n=2)

A: DiMeF(9,5)

B: DiMeF(11,3)

C: DiMeF(11,5) high concentrations

D: DiMeF(11,5) low concentrations

Several methods for the quantitative determination of FFA have been published. In these methods the lipids have to be pre-fractionated to obtain a FFA-enriched fraction (see *Stansby et al.,* 1990 and the literature cited therein). Besides the fact that all these methods are time-consuming, the risk for losses or formation of artefacts have to be considered. Recently, methods for the direct identification or determination of FFA have been reported. They require, however, the use of unusual procedures and facilities such as multidimensional GC-MS (*Wahl et al.,* 1995) or HPLC-GC with a FID-PID (photo ionisation detector) system (*Boselli et al.,* 2000). The advantages of the ion trap GC-MS/MS technique as described in the present work are manifold. It allows the determination of FFA in the methyl ester extract of plant lipids in the presence of co-eluates without prior separation, and it is fast and sensitive. The method using GC-MS/MS has successfully been applied to determine DiMeF in green tea and in dried herbs and vegetables (see chapter 4.1.2).

4.1.2 Furan fatty acid content of green tea and dried green herbs and vegetables

The contents of FFA in dried green plant material was determined as described in chapter 3.3.3 and chapter 3.4.2. Leek, chervil, dill and green tea were analysed in independent duplicates, denoted A and B. The amounts of FFA in the investigated samples are shown in Tab. 15. Within the group of pentyl FFA, two monomethyl substituted, two dimethyl substituted and two olefinic derivatives were identified in at least some of the samples. One propyl FFA was found exclusively in chive. The samples were also screened for FFA with a butyl, hexyl or heptyl side chain, based on the considerations in chapter 4.1.1. None of them were identified in any sample. The dimethyl substituted FFA were analysed quantitatively whereas the monomethyl substituted FFA could only be analysed qualitatively because of lack of standard substances. MeF(9,5) was found in green tea, dill and leek, whereas MeF(11,5) was present in green tea, chervil and chive. To our knowledge no data on the FFA composition of tarragon, basil, savory, chervil, dill, onion and leek are available in the literature.

The amounts of the pentyl DiMeF differed considerably in the investigated herbs and vegetables. In general, DiMeF(11,5) was present in higher amounts compared to DiMeF(9,5), which is similar to the findings by *Hannemann et al.* (1989).

Table 15: Concentrations of furan fatty acids in green tea, different herbs and vegetables

sample	DiMeF(11,3)[1]	MeF(9,5)	MeF(11,5)	DiMeF(9,5)[1]	DiMeF(11,5)[1]	DiMeF(11, 5:1)[2]	DiMeF(11:1, 5)[2]
tarragon	n.d.[3]	n.d.	n.d.	0.6	111.1	5.1	4.3
basil	n.d.	n.d.	n.d.	n.d.	7.5	tr.[4]	tr.
savory	n.d.	n.d.	n.d.	n.d.	2.5	tr.	n.d.
chervil A	n.d.	n.d.	d.[5]	8.4	97.1	7.6	13.5
chervil B	n.d.	n.d.	d.	7.1	107.8	13.4	14.8
dill A	n.d.	d.	n.d.	tr.	35.2	25.9	23.5
dill B	n.d.	d.	n.d.	3.6	11.5	n.d.	n.d.
chive	1.7	n.d.	d.	tr.	18.9	5.2	7.1
onion	n.d.	n.d.	n.d.	n.d.	0.6	tr.	tr.
leek A	n.d.	d.	n.d.	n.d.	28.1	tr.	tr.
leek B	n.d.	d.	n.d.	n.d.	32.9	tr.	tr.
green tea A	n.d.	d.	d.	6.7	241.9	1.4	1.7
green tea B	n.d.	d.	d.	9.5	335.3	2.0	2.7

1. µg/g dry matter
2. peak area ratio to DiMeF(11,5) in %
3. not detected
4. traces
5. detected

DiMeF(11,5) was found in all samples, whereas DiMeF(9,5) could not be detected in savory, basil, leek and onion. Chervil and tarragon were shown to be rich (> 100 µg/g) in DiMeF(11,5), whereas in onion very small amounts (< 1 µg/g) of this FFA were detected. These findings are in agreement with those of *Hannemann et al.* (1989), who claimed that FFA occur in higher amounts in the green parts of plants than in the trunks, roots and seeds. However, the values obtained here were approximately ten times lower when compared to results published for dried parsley (*Masanetz and Grosch*, 1998) and dried spinach (*Masanetz et al.*, 1998).

Chive was shown to contain 18.9 µg/g DiMeF(11,5) which agrees quite well with the 16 µg/g found by *Hannemann et al.* (1989). The content of DiMeF(11,3) in chive was very low (1.7 µg/g); *Hannemann et al.* (1989) detected this propyl DiMeF in small amounts (< 4 µg/g) in grasses (blade), clover, birch (leaf), wheat and potato (leaf), but not in chive. In general, FFA with a propyl side chain are supposed to occur only in minute amounts in land plants. High amounts (145 µg/g) of DiMeF(11,3) were found by *Hannemann et al.* (1989) in the algae *Chlorophyta spec*.

The amounts of DiMeF(9,5) and DiMeF(11,5) found in green tea were slightly higher than those published by *Guth and Grosch* (1993a). The amount and composition of the FFA in green tea probably depend on provenience and variety. Processing of the green tea and further treatment could also have an influence on both amount and composition of the FFA. Depending on the kind and care of processing, degradation and transformation of the FFA can be more or less advanced. In this context, *Guth and Grosch* (1993a) observed a 10-fold amount of pentyl DiMeF in fresh green tea leaves compared to green tea powder.

The origin of the olefinic derivatives of the DiMeF(11,5) found in most of the investigated samples is not fully understood. According to *Ishii et al.* (1988a), olefinic FFA are artefacts formed during sample preparation. DiMeF(9, 5:1) and DiMeF(9:1, 5) were found to be generated during the analytical process (GC analysis with split-splitless injection) from a diketoene formed by autoxidation of the FFA. The olefinic FFA found in the samples could have been formed from diketoenes already present in the material as well. However, *Boselli et al.* (2000) did not agree with the artefact-assumption for DiMeF(11, 5:1) and DiMeF(11:1, 5) but claimed these olefinic FFA to be of natural origin in the investigated oil samples.

4.2 Isolation of furan fatty acid photooxidative degradation products by using micro simultaneous distillation solvent extraction

In order to study the aroma active furan fatty acid photooxidative degradation products, it was first necessary to choose a suitable method for the isolation of the volatile compounds from the heterogenous plant material. As has been described by *Parliment* (1997), sample preparation for the analysis of food aroma is complicated by a number of factors. The generally low concentration level, the complexity, the variation of volatility and the instability of the aroma compounds as well as the complex food matrices could create problems during isolation procedures. Various isolation techniques have been developed (see e.g. in *Schreier* (1984), *Maarse and Grosch* (1996)). However, the sample preparation remains the most critical step in the entire analytical process of the investigation of volatile substances (*Schreier*, 1984) and has to be carefully adapted to the corresponding problem.

A modified microversion (*Godefroot et al.*, 1981) of the simultaneous distillation solvent extraction (SDE) apparatus described by *Likens and Nickerson* (1964) was used for the isolation of the aroma compounds pentanal, 2,3-butanedione, 2,3-octanedione, MND, bovolide and dihydrobovolide (subsequently named as analytes). In combination with the applied OC injection mode, the micro-SDE allowed the direct GC analysis of the extract without any concentration step.

Since steam distillation can cause thermal reactions (*Garneo*, 1977), artefacts can arise from chemical degradation or reactions between the individual volatiles during the isolation procedure (*Schreier*, 1984). To ensure that the analytes were not formed during the micro-SDE, a solution of pentyl DiMeF was subjected to this distillation procedure (see chapter 3.4.1). GC-MS analysis of this isolate showed that no aroma compounds were formed during the distillation. Distillation of a mixture of the analytes by micro-SDE did not show an alteration of the analytes either. Herewith no artefact formation was caused using the micro-SDE procedure.

Recovery studies with the analytes were performed to evaluate the efficiency of the sample preparation using micro-SDE. The recoveries (RV) were determined both from

an aqueous mixture and from a green tea matrix. In Tab. 16 the RV of the analytes and of the ethyl decanoate (ISTD 1) and the ethyl valerate (ISTD 2) from the two different media are shown. The RV are reported as percent of the initial peak area ratio of the analytes to the internal standard methyl undecanoate (ISTD 3).

Table 16: Recoveries (RV) and relative standard deviations (RSD) of the aroma active volatile furan fatty acid degradation products and of two ethyl esters extracted by micro-SDE from an aqueous model mixture and from a green tea matrix

	aqueous mixture		green tea matrix	
analyte	RV [%][1]	RSD [%]	RV [%][2]	RSD [%]
pentanal	94.5	4.8	47.3	8.0
2,3-butanedione	83.6	3.2	33.6	5.3
2,3-octanedione	94.5	2.4	76.6	6.4
MND	101.0	4.3	89.2	3.8
bovolide	92.3	2.4	74.4	8.4
dihydrobovolide	76.5	7.6	35.3	12.0
ethyl decanoate (ISTD 1)	91.0	3.6	81.1	6.1
ethyl valerate (ISTD 2)	101.6	2.5	92.0	4.9

1. mean value (n = 5)
2. mean value (n = 6)

The RV of the compounds extracted from the aqueous model mixture reflect the optimal conditions for the micro-SDE method. The reproducibility of the procedure is satisfactory with a relative standard deviation of less than 5 % (with one exception). Most of the analytes were extracted at a high percentage of > 90 %. The low RV of 2,3-

butanedione (83.6 %) was expected because of the relatively high volatility of this compound compared to the other analytes. In contrast to 2,3-butanedione, the observed RV of dihydrobovolide (76.5 %) is probably due to its low volatility in steam. Experiments with a reduced distillation time of 50 % revealed an incomplete extraction of both bovolides. However, a considerable extension of the used distillation time (60 min) would increase the risk of unacceptable losses of the other compounds.

The two ethyl esters (ISTD 1, ISTD 2) were used in subsequent analyses as internal standards and as reference substances in a controlling system to evaluate the micro-SDE process. The RV of both substances (> 90.0 %) was satisfactory, and the reproducibility was very good. ISTD 2 was directly added to the organic solvent. The RV of 101.6 % indicates an optimal, loss-free recycling of the organic solvent. In addition to an incomplete extraction from the water phase, a part of the lower RV could only be due to slight losses of the analytes in the circular flow of the water phase.

The RV of the aroma compounds isolated from a slurry with green tea were much lower compared to the ones obtained in the model experiment with the aqueous mixture. The relative standard deviations (RSD) varied between 3.8 % (MND) and 12.0 % (dihydrobovolide), indicating reproducible losses during the micro-SDE procedure. The RV of both ethyl esters, ISTD 1 added to the slurry and ISTD 2 added to the organic solvent, made clear that some losses occurred in both circular flows. The RV of ISTD 1 and ISTD 2 could be taken as indicators for losses of the aroma compounds from these phases as well. The low RV of pentanal (47.3 %), 2,3-butanedione (33.6 %) and dihydrobovolide (35.3 %), however, can not be explained by losses due to the micro-SDE procedure alone. It is very likely that the matrix of the green tea influences the distillation of the analytes to a different extent.
Despite the low RV of some aroma compounds from the green tea sample the used micro-SDE was estimated to be suitable because of its reproducibility. However, one must be aware that the yield of extraction of certain compounds can vary within large ranges depending on the food sample.

The GC analysis of the compounds extracted by micro-SDE allowed a semi quantitative evaluation. The results were expressed as peak area ratio to an internal standard. Ethyl

decanoate (ISTD 1) was shown to be suitable for both sample preparation and GC analysis. However, it must be taken into account that by using only one ISTD the self-compensating effect of gain or losses during the extraction procedure can not be the same for all analytes because of their different chemical and physical properties. In order to ensure reproducible conditions for sample preparation (and with it constant starting conditions for the semi quantitative analysis), it was necessary to have the ISTD 1 in a constant concentration in the extract. To verify this requirement, a controlling system was developed using two additional standards (ISTD 2 and ISTD 3). Ethyl valerate (ISTD 2) was added to the organic phase prior to the isolation procedure, and methyl undecanoate (ISTD 3) was added to the extract after the isolation procedure. In Tab. 17 the mean values (MV) and the RSD of the peak area ratios (R_{MS}) of ISTD 1 and ISTD 2 to ISTD 3 are shown for different series of measurements, consisting of 6 or 9 green tea extracts from the light exposure experiments (see chapter 4.4).

Table 17: Determination of the reproducibility of the micro-SDE procedure using green tea samples. Peak area ratios (R_{MS}) for ethyl decanoate and ethyl valerate to the internal standard methyl undecanoate and corresponding relative standard deviations

	R_{MS} (ISTD 1/ISTD 3)		R_{MS} (ISTD 2/ISTD 3)	
series of measurements	MV[1]	RSD[2] [%]	MV	RSD [%]
n[3] = 9	0.624	4.8	0.336	5.0
n = 6	0.612	7.6	0.320	6.7
n = 6	0.627	2.8	0.343	6.3
n = 9	0.694	4.0	0.384	6.0
n = 6	0.719	3.1	0.391	4.8
n = 6	0.591	5.6	0.359	6.7

1. mean value
2. reproducibility expressed as relative standard deviation (RSD)
3. number of analyses

The calculated R_{MS} values for ISTD 1/ISTD 3 and ISTD 2/ISTD 3 showed good RSD from 2.8 % to 7.6 % and from 4.8 % to 6.7 %, respectively. These R_{MS} values were used as indicators for the concentrations of ISTD 1 and ISTD 2 in the extracts. The low RSD for all sets of experiments made clear that the concentrations of ISTD 1 and ISTD 2 in the extract were constant, and consequently the micro-SDE process was reproducible. Additionally, with these two ratios, circulation related problems of the organic and/or aqueous solution during the micro-SDE procedure could be revealed. Depending on the losses, the yield of one or both ISTD was affected. In such cases it had to be assumed that the yield of the aroma compounds was also unacceptably affected and the extraction had to be repeated.

The use of three ISTD turned out to considerably enhance the quality assurance for the sample preparation by micro-SDE. Provided that the starting material was homogeneous, information regarding the reproducibility without making replicate analyses could be obtained.

4.3 Oxidative stability of furan fatty acid photooxidative degradation products

Little is known about the oxidative stability of aroma active FFA photooxidative degradation products. *Oechslin* (1997) showed that bovolide and dihydrobovolide are stable under photooxidative conditions. In the present study the photooxidative stability of the two diones MND and 2,3-octanedione was investigated. Degradation of these two aroma compounds could lead to other aroma compounds that significantly contribute to the flavour of food. Therefore the main attention was paid to the light induced formation of aroma compounds in the oxidative degradation model experiments with MND and 2,3-octanedione.

4.3.1 3-Methyl-2,4-nonanedione

The experiments were carried out in hexane and in methanol as described in chapter 3.6. The main products obtained in the two solvents under the different experimental conditions are listed in Tab. 18 and 19.

Table 18: Main products derived from MND under different reaction conditions in hexane, expressed as peak area ratio to the internal standard ethyl decanoate (ISTD 1)

peak no.	RI	compound	identification[1]	photo-oxidation	dark oxidation	unsensitised oxidation	photochemical reaction
1	986	2,3-butanedione	r.s.	2.0	n.d.[2]	4.6	n.d.
2	1121	ISTD 2	r.s	0.2	0.2	0.2	0.2
3	1321	2,3-octanedione	r.s	5.1	0.1	7.2	tr.[3]
4	1449	acetic acid	r.s	0.7	n.d.	1.1	n.d.
5	1583	unknown[4]		0.5	4.1	0.3	5.6
6	1643	ISTD 1	r.s	1.0	1.0	1.0	1.0
7	1727	HMND	MS, IR	11.4	tr.	16.3	n.d.
8	1741	MND	r.s	46.3	102.7	47.5	43.5
9	1861	hexanoic acid	r.s	5.1	n.d.	7.6	tr.
10	1935	BHT	r.s	-	-	-	-
11	1996	unknown[4]		0.7	n.d.	1.3	n.d.
12	2009	unknown[4]		0.4	n.d.	0.5	n.d.
13	2270	unknown[4]		2.4	n.d.	2.8	n.d.

1. Identification based on the following criteria: r.s.: RI and mass spectra consistent with reference substances; MS: identification based on mass spectral characteristics; IR: identification based on infrared spectral characteristics.
2. not detected
3. traces
4. Mass spectral characteristics see Tab. 20

Table 19: Main products derived from MND under different reaction conditions in methanol, expressed as peak area ratio to the internal standard ethyl decanoate (ISTD 1)

peak no.	RI	compound	identification[1]	photooxidation	dark oxidation
1	986	2,3-butanedione	r.s.	2.6	0.3
2	1068	unknown[2]		0.2	0.1
3	1129	ISTD 2	r.s	0.2	0.2
4	1189	methyl hexanoate	MS	1.3	0.3
5	1323	2,3-octanedione	r.s	4.8	0.7
6	1443	acetic acid	r.s	0.5	n.d.[3]
7	1577	unknown[2]		1.9	0.2
8	1638	ISTD 1	r.s	1	1
9	1722	HMND	MS, IR	17.1	0.6
10	1740	MND	r.s	35	63.4
11	1859	hexanoic acid	r.s	2.7	n.d.
12	1935	BHT	r.s	-	-
13	1947	unknown[2]		2.0	n.d.

1. Identification based on the following criteria: r.s.: RI and mass spectra consistent with reference substances; MS: identification based on mass spectral characteristics; IR: identification based on infrared spectral characteristics.
2. mass spectral characteristics see Tab. 20
3. not detected

Only products with an R_{MS} of ≥ 0.1 (based on TIC) were considered. MND showed to be stable under oxidative conditions in the absence of light (dark oxidation). No significant decrease of the dione was observed neither in hexane nor in methanol as compared to the experiments with light exposure. The GC-MS chromatogram of the products of MND formed in hexane under photooxidative conditions for 24 h is shown in Fig. 15, and the corresponding chromatogram in methanol as solvent in Fig. 16, respectively.

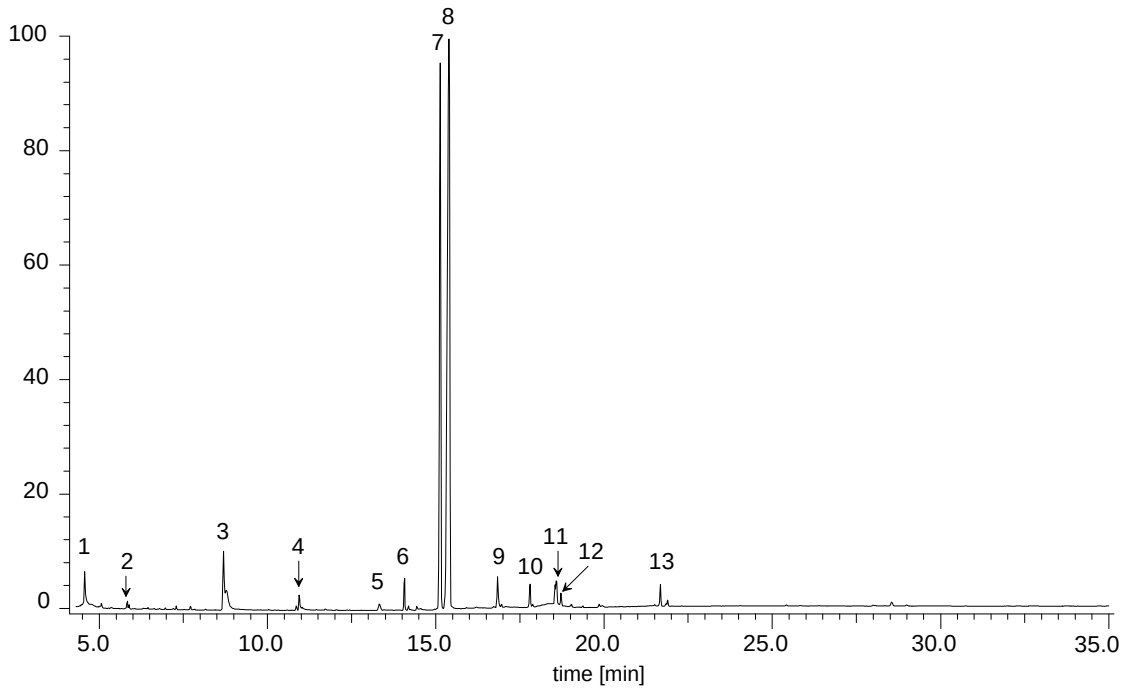


Figure 15: GC-MS chromatogram of products derived from MND under photooxidative conditions in hexane (see Tab. 18)

In both cases only few products were formed. The solvent had little influence on the product composition. Product type and amount differed only slightly. In both solvents the same five products were found. Among these compounds the four well known aroma compounds 2,3-butanedione, 2,3-octanedione, acetic acid and hexanoic acid were identified.

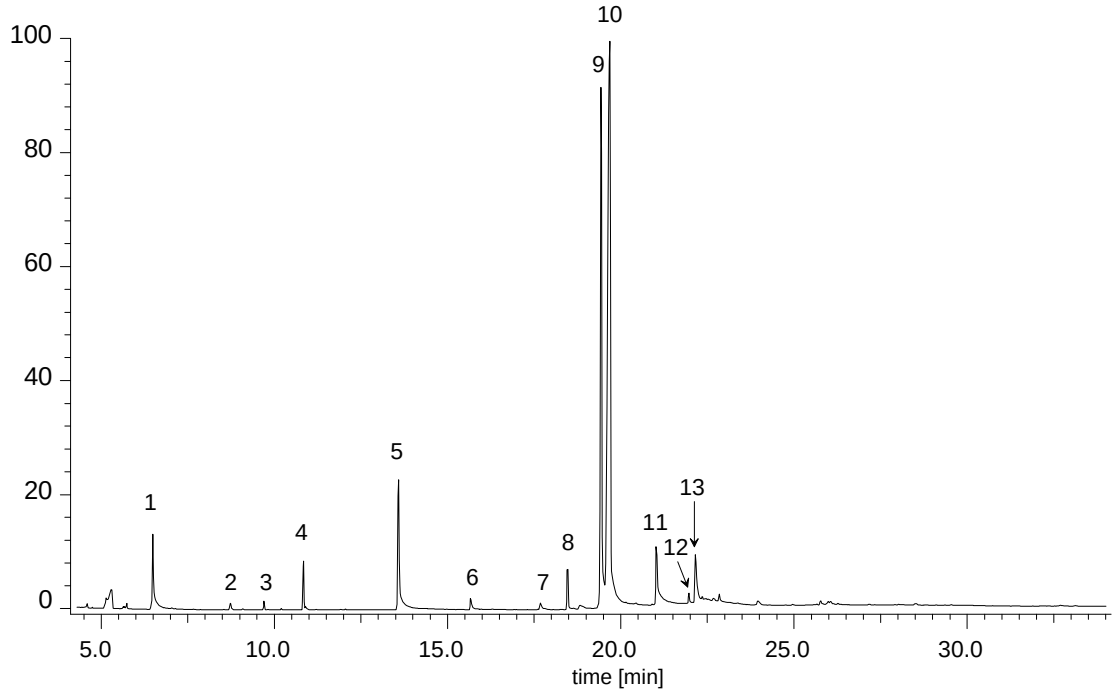


Figure 16: GC-MS chromatogram of products derived from MND under photooxidative conditions in methanol (see Tab. 19)

The main oxidation product of MND was 3-hydroxy-3-methyl-2,4-nonanedione (HMND). This substance was shown to be aroma active by using GC-O; the odour description was rubbery, earthy and plastic-like. The compound was identified based on the mass spectrum (GC-MS) and the vapour phase infrared spectrum (GC-IR). The mass spectrum of HMND is shown in Fig. 17. The molecular ion was not observed. The fragment ions at m/z 43, 71 and 99 are formed by α-cleavage, whereas the fragment ions at m/z 88 and 144 are attributed to McLafferty rearrangements.

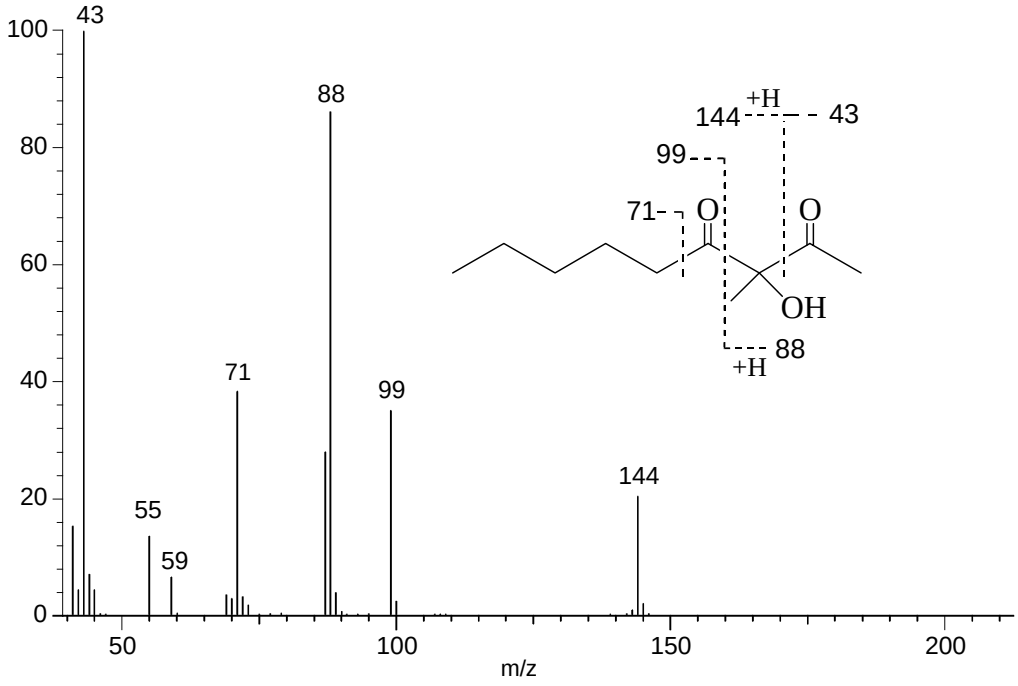


Figure 17: GC-MS spectrum of HMND

The infrared spectra of HMND and of MND are shown in Fig. 18. According to *J.H. van der Maas* (2002, personal communication) the two spectra can be interpreted based on the spectra of similar molecules as follows: The spectra of MND and HMND show both a C=O stretching band at 1714 cm^{-1}. For comparison the IR spectrum of 3,3-dimethyl-2,4-pentadione (neat, *Sadtler 56098*) shows a doublet with maxima at ~1720 cm^{-1} and ~1700 cm^{-1}, but in this molecule the carbonyl groups are trans oriented. The splitting in the spectrum of 3,3-dimethyl-2,4-pentadione is probably due to a field effect (out-of-phase and in-phase coupling). For HMND the OH stretching band of a free (tertiary) hydroxyl group can be expected at about 3615 cm^{-1}. Evidently, the hydroxyl group exhibiting at 3478 cm^{-1} in the spectrum of HMND (Fig. 18 B) is hydrogen bonded to a carbonyl group. As a consequence its stretching frequency is lowered and the peak is broadened. The O···H–O angle in a 5-membered ring is ~120°. The frequency of the C=O stretching band, however, may remain unaltered, since in a 5-membered ring the C=O···H angle is roughly 90° (hydrogen bond orthogonal to the carbonyl group). The C=O stretching bands in the spectra of 1-hydroxy-propanone (neat, *Aldrich 13,818-5*) and 3-hydroxy-2-butanone (neat, *Aldrich 10,897-9*) are found at 1724 and 1716 cm^{-1}. These data clearly show that intramolecular hydrogen bonding does not affect the C=O

stretching. Therefore MND and HMND exhibit the C=O stretching peak in the same region.

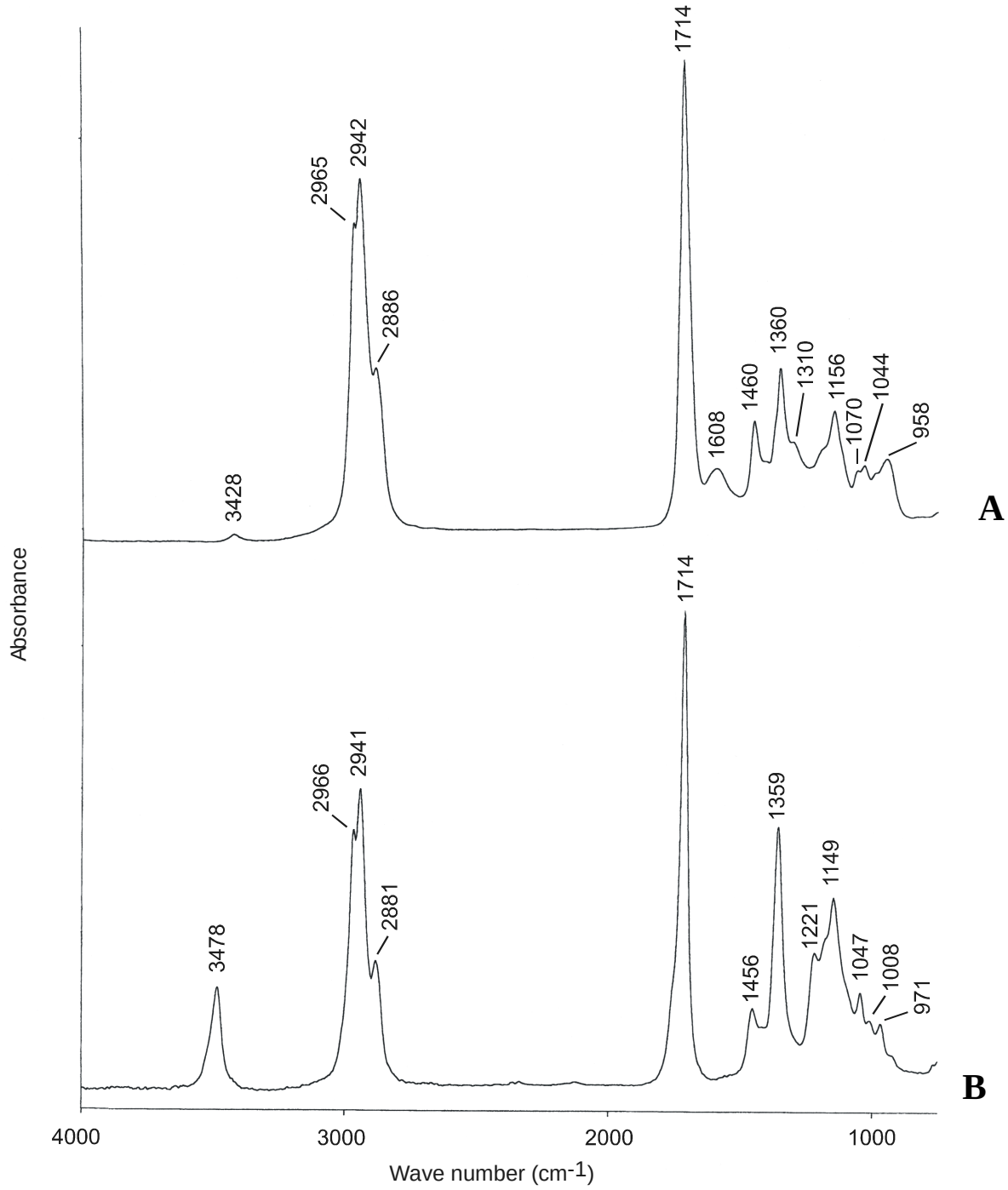


Figure 18: GC-IR spectra of MND (A) and HMND (B). For interpretation see text.

In MND, keto-enol tautomerism may occur. The broad band at 1608 cm^{-1} in the spectrum of MND (Fig. 18 A) could be due to this phenomenon, but no band is present in the OH stretching region of the spectrum. 3-Methyl-2,4-hexanedione (neat, *Sadtler 56100*) shows a carbonyl doublet as 3,3-dimethyl-2,4-pentadione (neat, *Sadtler 56098*) (same pattern, ~ 5 cm^{-1} red-shifted), the broad 1600 cm^{-1} band and no clear OH stretching band in the stretching region as well. However, there is some evidence in the spectrum of 3-methyl-2,4-hexanedione that points to the presence of hydrogen bonding. Compared to the spectrum of 3,3-dimethyl-2,4-pentadione (neat, *Sadtler 56098*), bands are here less pronounced, and an appreciable background between 1800 and 900 cm^{-1} is observed. The presence of some hydrogen bonding, and the absence of an OH stretching band in the stretching region in the spectrum of MND can be accepted provided that (1) the keto-form is the dominant one and (2) the OH stretching band is very broad. Furthermore the hydroxyl group has an enolic (more acidic) character. To sum up the interpretation by van der Maas the spectra shown in Fig. 18 are consistent with the structures of MND and HMND, respectively.

The formation of a hydroxylated dione as oxidation product of MND is supported from literature reports as well. Studies by *House and Gannon* (1958) have shown that the oxidation of enolisable β-diketones with peracids leads to α-hydroxy-β-diketones. Furthermore, in the reaction of enolisable β-diketones with singlet oxygen, the formation of α-hydroxy-β-diketones has been reported by *Yoshioka et al.* (1998).

A compound with the same mass spectral characteristics as HMND has already been mentioned by several authors. *Horita and Hara* (1986) described an unknown compound in an investigation on aroma concentrates of green tea after exposure to light. *Braggins* (1996) reported about an unknown odour compound of rendered sheep fat which elutes in the same region as HMND under comparable chromatographic conditions and showed identical mass spectral characteristics. The odour of the compound was described by *Braggins* (1996) as plastic-like. The fact that HMND is a photooxidative and autoxidative degradation product of pentyl DiMeF was first recognised by *Pompizzi* (1999) who also reported this compound to be a Baeyer-Villiger oxidation product of MND. However, the provisional assignment as an ester made by *Pompizzi* (1999) was not correct, and has been revised by the present study.

Some of the products formed under the different experimental conditions have not been identified yet. The mass spectral characteristics of these unknown compounds are shown in Tab. 20.

Table 20: Mass spectral characteristics of unknown products derived from MND under different reaction conditions in hexane (Tab. 18) and methanol (Tab. 19)

RI	solvent	fragment ion (intensity)
1068	methanol	43 (80), 57 (100), 71 (60), 85 (50)
1577	methanol	43 (100), 55 (20), 71 (65), 99 (90)
1583	hexane	43 (75), 71 (20), 85 (100), 100 (60), 113 (20)
1947	methanol	43 (100), 71 (50), 86 (20), 99 (70), 118 (20), 131 (30)
1996	hexane	43 (50), 99 (100), 114 (50), 170 (20)
2009	hexane	43 (70), 71 (30), 88 (100), 99 (25), 130 (20), 144 (25), 186 (20)
2270	hexane	43(40), 71 (50), 99 (100), 114 (20), 170 (20)

In control experiments it was shown that HMND, 2,3-butanedione, 2,3-octanedione, acetic and hexanoic acid are only formed under photooxidative conditions. Without light exposure (Tab. 18 and 19, dark oxidation), these compounds were either absent or present in minute amounts only. This is also valid for the unknown compounds listed in Tab. 18 and 19, with one exception. The unknown compound with RI 1583 formed by oxidation of MND in hexane (Tab. 18, peak no. 5) is present in much higher amounts in the dark oxidation compared to the photooxidation.

The influence of the sensitiser on product formation (unsensitised oxidation) as well as the products formed in absence of oxygen (photochemical reaction) were investigated in hexane as solvent (Tab. 18). No difference between unsensitised oxidation and photooxidation was observed. This result was surprising because it was assumed that

MND can not play the role of a sensitiser. Its absorbance maximum is at 287 nm and the lamps used for the experiments under photooxidative conditions scarcely emit light at this wavelength. This result is in contrast to the statement by *Matsuura and Saito* (1976) who claimed that photooxidation without sensitiser generally gives a more complex mixture of oxidation products than photooxidation with sensitiser.

In the photochemical experiment (Tab. 18) trace amounts of 2,3-octanedione and hexanoic acid were detected, the other compounds formed under photooxidative conditions were not found. Astonishingly, the unknown compound with RI 1583 (Tab. 18, peak no. 5) was formed in similar amounts as in the dark oxidation. This remains to be explained.

Following *Yoshioka et al.* (1998) the formation of HMND can be explained through the ene reaction of the enol form with singlet oxygen to give the α-hydroperoxy-β-diketone. This substance is subsequently cleaved to the corresponding α-hydroxy-β-diketone. The formation of 2,3-butanedione, 2,3-octanedione, acetic and hexanoic acid can formally be explained by the oxidation of the enolic double bonds of the two most favourable enol forms of MND (Fig. 19). Oxidation of the 2,3-enol leads to acetic acid and 2,3-octanedione, whereas oxidation of the 3,4-enol results in hexanoic acid and 2,3-butanedione.

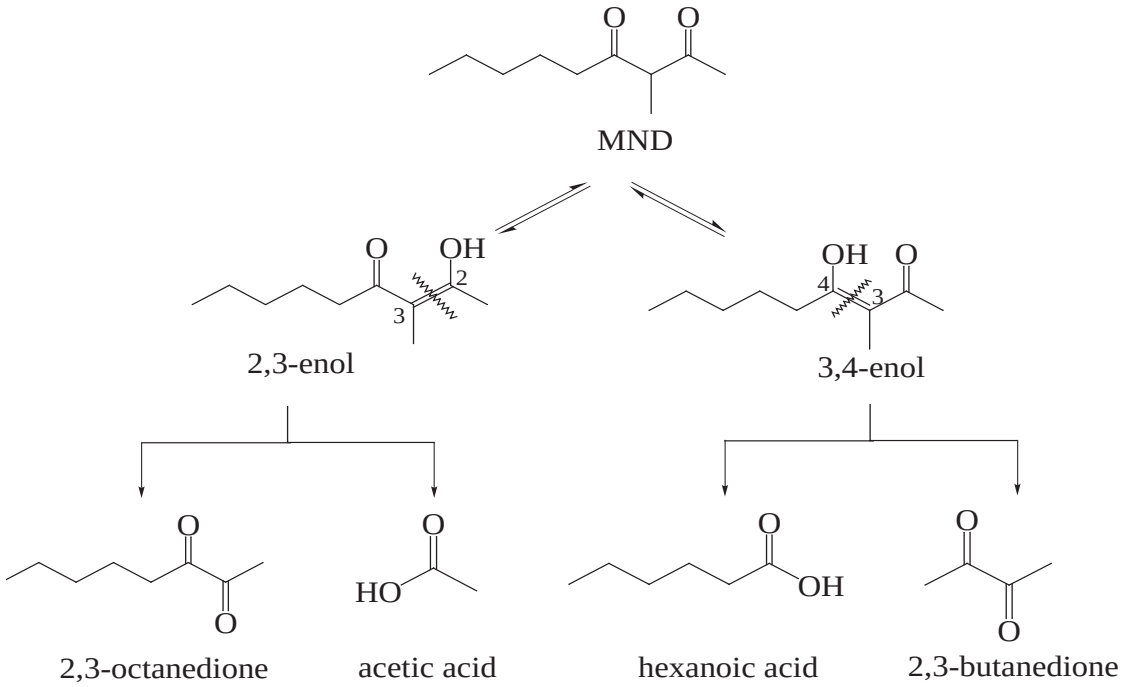


Figure 19: Proposed oxidation of the 2,3- and 3,4-enol forms of 3-methyl-2,4-nonanedione (MND) to 2,3-octanedione, acetic acid, hexanoic acid and 2,3-butanedione

Based on the results obtained in the present study the formation of 2,3-octanedione can not only be explained by the pathway starting from pentyl DiMeF suggested by *Pompizzi* (1999), but also by a pathway in which MND is formed as an intermediate.

4.3.2 2,3-Octanedione

The experiments were carried out in hexane and methanol as described in chapter 3.6. The main products obtained with the two solvents are listed in Tab. 21 and 22. Only products with an R_{MS} (based on TIC) of ≥ 0.1 were considered. The GC-MS chromatogram of the products of 2,3-octanedione formed in hexane under photooxidative conditions for 24 h is shown in Fig. 20 and the corresponding chromatogram in methanol as solvent in Fig. 21, respectively.

Table 21: Main products derived from 2,3-octanedione under different reaction conditions in hexane, expressed as peak area ratio to the internal standard ethyldecanoate (ISTD 1)

peak no.	RI	compound	identification[1]	photo-oxidation	dark oxidation	unsensitised oxidation	photochemical reaction
1	998	pentanal	r.s.	0.2	n.d.[2]	0.6	n.d.
2	1052	impurity of solvent	r.s	4.7	n.d.	8.1	11.9
3	1069	impurity of solvent	r.s	4.7	n.d.	9.2	14.3
4	1120	ISTD 2	r.s	0.2	0.2	0.2	0.2
5	1138	unknown[3]		0.3	n.d.	19.9	1.4
6	1271	unknwon[3]		0.5	n.d.	n.d.	n.d.
7	1320	2,3-octanedione	r.s.	17.8	48.1	6.8	41.6
8	1452	acetic acid	r.s	1.5	n.d.	4.9	1.5
9	1507	unknown[3]		0.4	n.d.	n.d.	n.d.
10	1600	unknown[3]		0.4	n.d.	2.6	4.4
11	1647	ISTD 1	r.s.	1.0	1.0	1.0	1.0
12	1857	hexanoic acid	r.s.	16.0	n.d.	51.0	31.4
13	1932	BHT	r.s.	-	-	-	-

1. Identification based on the following criteria: r.s.: RI and mass spectra consistent with reference substances;
2. not detected
3. Mass spectral characteristics see Tab. 23

Table 22: Main products derived from 2,3-octanedione under different reaction conditions in methanol, expressed as peak area ratio to the internal standard ethyl decanoate (ISTD 1)

peak no.	RI	compound	identification[1]	photooxidation	dark oxidation
1	993	pentanal	r.s.	0.4	n.d.[2]
2	1124	ISTD 2	r.s	0.2	0.2
3	1163	methyl hexanoate	MS	0.7	0.1
4	1320	2,3-octanedione	r.s	68.8	51.5
5	1441	acetic acid	r.s	0.4	n.d.
6	1643	ISTD 1	r.s	1.0	1.0
7	1854	hexanoic acid	r.s	2.2	n.d.
8	1931	BHT	r.s	-	-

1. Identification based on the following criteria: r.s.: RI and mass spectra consistent with reference substances; MS: identification based on mass spectral characteristics;
2. not detected

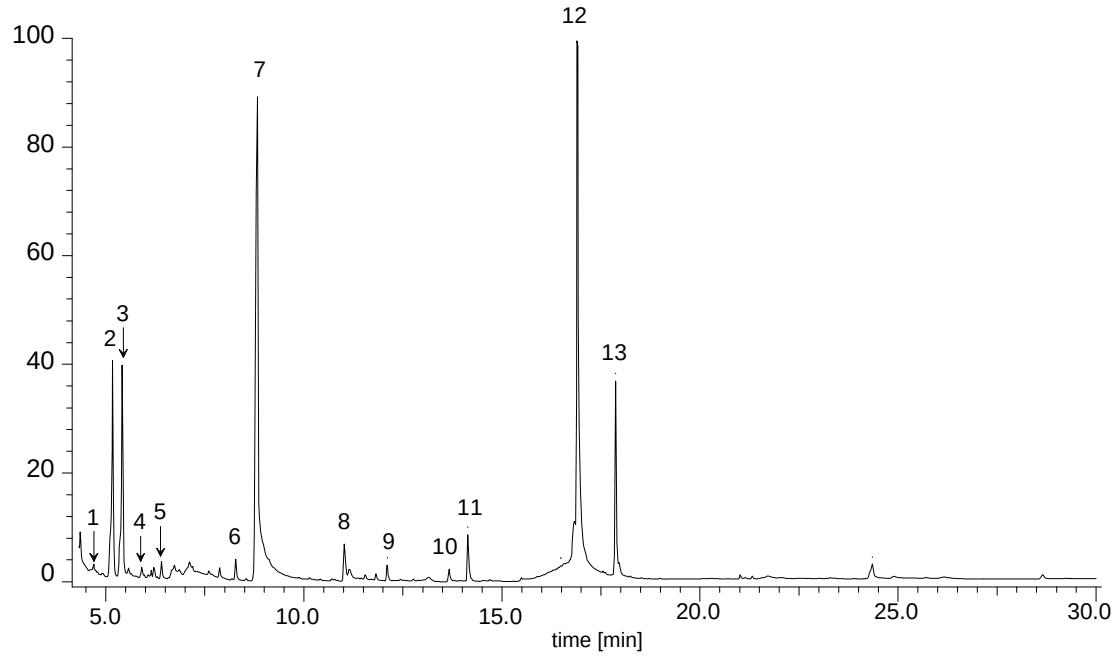


Figure 20: GC-MS chromatogram of products derived from 2,3-octanedione under photooxidative conditions in hexane (see Tab. 21)

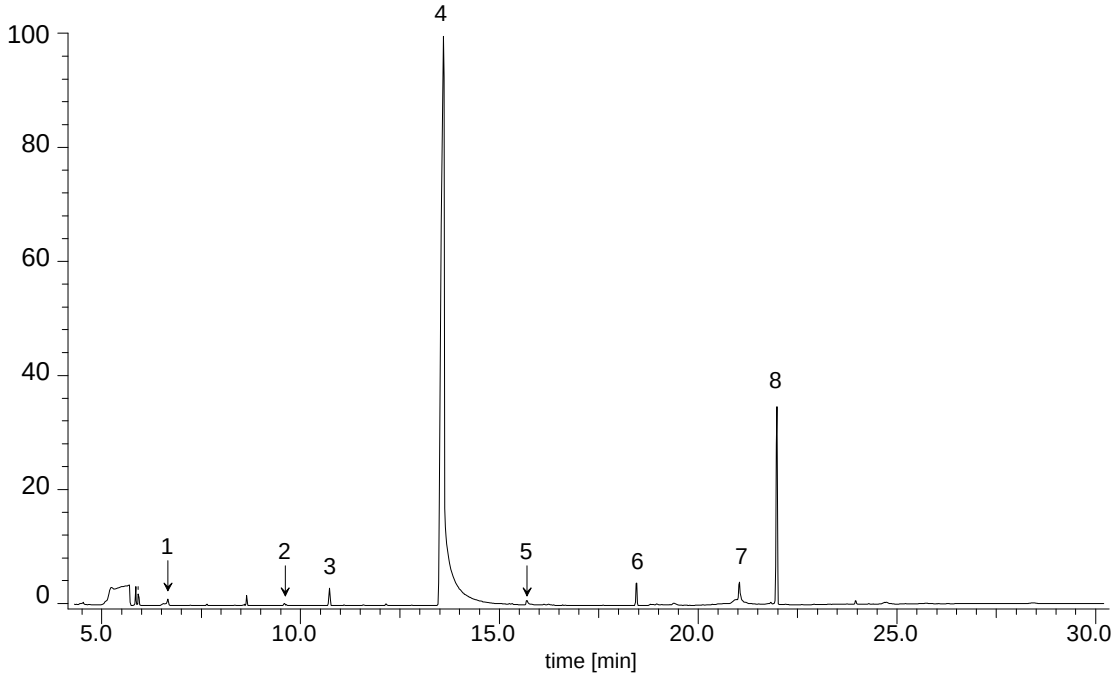


Figure 21: GC-MS chromatogram of products derived from 2,3-octanedione under photooxidative conditions in methanol (see Tab. 22)

As in the experiments with MND (chapter 4.3.1), only few products were formed and the solvent had little influence on the product composition. Pentanal, acetic acid and hexanoic acid were identified both in hexane and in methanol. Additionally in hexane four unknown products were formed and in methanol one compound which was tentatively identified as methyl hexanoate was present. The mass spectra of the unknown compounds are listed in Tab. 23. All these products were absent in the control experiment (dark oxidation) with the exception of methyl hexanoate, which was present in small amounts.

Table 23: Mass spectral characteristics of unknown products derived from 2,3-octanedione under different reaction conditions in hexane (Tab. 21)

RI	solvent	fragment ion (intensity)
1138	hexane	43 (20), 71 (100), 115 (60)
1271	hexane	43 (70), 56 (30), 57 (20), 69 (20), 84 (100), 85 (30)
1507	hexane	43 (100), 71 (20), 99 (40)
1600	hexane	43 (100), 58 (30), 71 (20), 86 (50)

The influence of the sensitiser on product formation (unsensitised oxidation) as well as possible products formed in absence of oxygen (photochemical reaction) were investigated in hexane as solvent (Tab. 21). As already observed in the experiments with MND, the unsensitised oxidation gave the same products as the photooxidation. Only the two unknown products with RI 1271 (Tab. 21, peak no. 6) and with RI 1507 (Tab. 21, peak no. 9) were not detected. All other products were formed in even higher amounts in absence of a sensitiser. A sensitiser activity of 2,3-octanedione must be taken into consideration. The absorption spectrum of 2,3-octanedione exhibits maxima at 270 nm and 430 nm. According to *Rubin* (1969), α-diketones absorb UV-light in the range from 270-300 nm and exhibit a second maximum at 330-540 nm due to n,π^* transitions. These diones may function as sensitisers.

In the photochemical experiment, the same compounds, except pentanal, were detected as in the unsensitised oxidation. According to *Chen and Ho* (1998) the mechanism of the unsensitised photoreaction with oxygen is similar to that of the photoreaction without oxygen.

The formation of pentanal can formally be explained by the oxidation of the enolic double bond of 2,3-octanedione. Oxidation of the 3,4-enol leads to pentanal and pyruvic acid (Fig. 22). However, the presence of pyruvic acid was not verified.

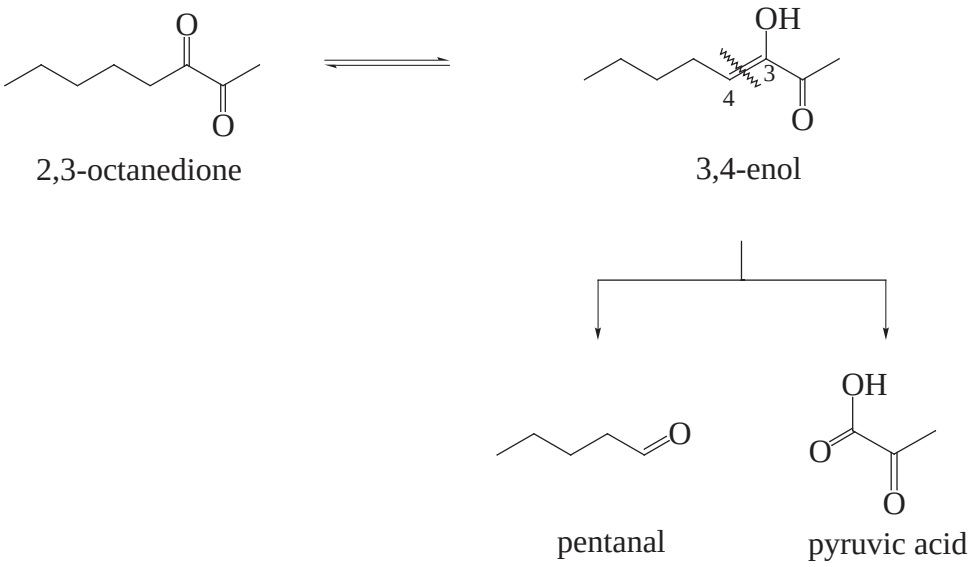


Figure 22: Proposed oxidation of the 3,4-enol form of 2,3-octanedione to pentanal and pyruvic acid

The main products of the light induced reactions were acetic acid and hexanoic acid. Carboxylic acids have been described in the literature as products of photoreactions of α-diketones (*Rubin*, 1969).

4.4 Formation of furan fatty acid photooxidative degradation products in green tea

The formation rate of the aroma compounds pentanal, 2,3-butanedione, 2,3-octanedione, MND, HMND, bovolide and dihydrobovolide was investigated in green tea as a representative of dried green plant material. As described in chapter 3.5 green tea was exposed to light for a time period of 20 and 25 days respectively, under different photooxidative conditions. The experiments were carried out at two illuminations (3‘200 lx and 40‘000 lx) in an air or oxygen atmosphere. As a control, the experiments were performed under the same conditions in the dark. In Fig. 23 the formation of pentanal and the two bovolides, and in Fig. 24 the formation of the different diones under the photooxidative conditions are shown in relation to the initial stage of the samples before light exposure.

All investigated aroma compounds increased during exposure to light, whereas no changes were observed in the samples stored in the dark. The formation curves of each compound in the three light exposure experiments were similar. 2,3-Butanedione showed the slightest relative change and only small differences between the three experiments. The other compounds followed two patterns. On the one hand, pentanal, 2,3-octanedione, bovolide and dihydrobovolide increased continuously during at least 15 days of light exposure. The most pronounced increase took place at the beginning of the experiment. On the other hand, MND and HMND reached a maximum content after two days of the light exposure, followed by a considerable decrease during the ongoing of the experiments. On the whole, the major changes occurred during the first two days of illumination. Light exposure experiments carried out in oxygen atmosphere gave higher relative changes when compared to the experiments performed in air at the same illumination.

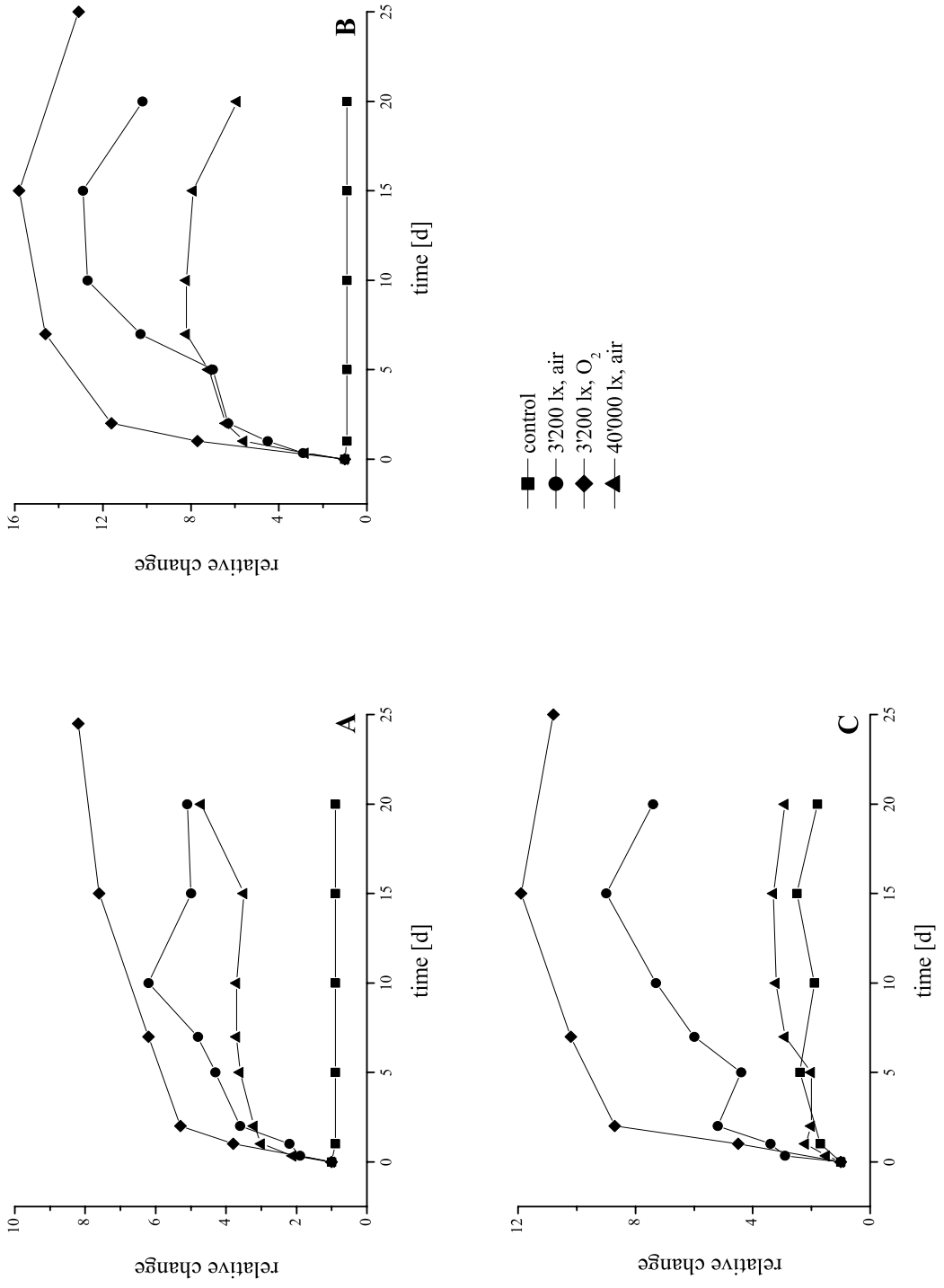


Figure 23: Formation of pentanal (A), bovolide (B) and dihydrobovolide (C) in green tea under photooxidative conditions

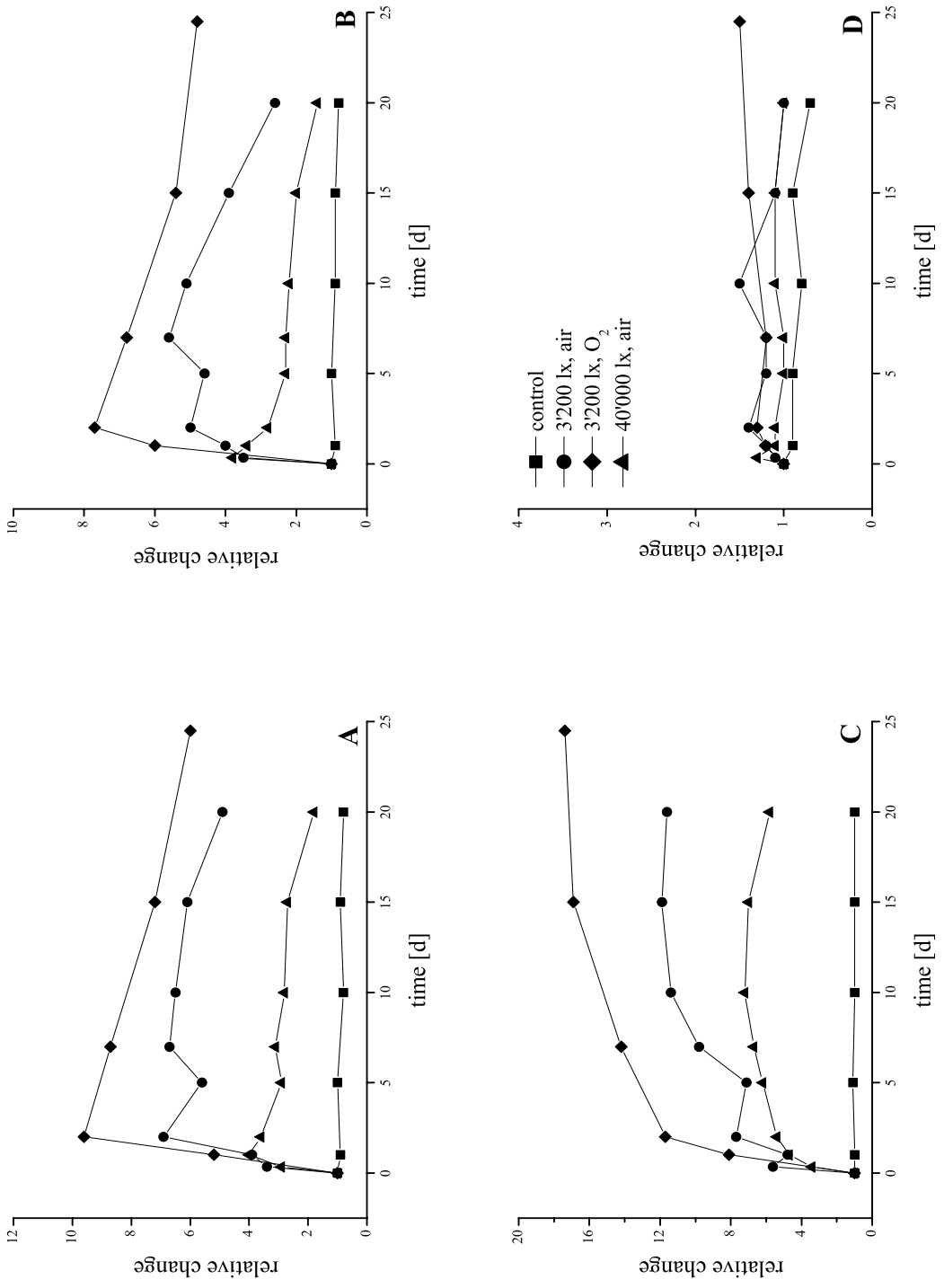


Figure 24: Formation of the diones MND (A), HMND (B), 2,3-octanedione (C) and 2,3-butanedione (D) in green tea under photooxidative conditions

An increase in the illumination from 3’200 lx to 40‘000 lx - all other experimental conditions were kept constant - did not result in an enhanced formation of FFA degradation products. On the contrary, less aroma compounds were formed. In addition, the maximum peaks for MND and HMND and the sharp increase for pentanal, bovolide and dihydrobovolide were already observed after one day of light exposure. *Sandmeier and Ziegleder* (1994) observed a similar effect in their investigations on photooxidation of chicken soup and broccoli soup powder. This unexpected behaviour can be explained with the type of light induced oxidation. Green tea contains chlorophyll that can act as sensitiser, thus the conditions for a photosensitised oxidation are given. It must be kept in mind that for this type of light induced oxidation the illumination does not have an influence on the product formation, and no induction phase is observed. In the present investigation both of these criteria were fulfilled. In samples which had been exposed to light for one day and subsequently stored in the dark for a period of 80 days, no change in the aroma compounds was measured (data not shown). This result further supports the evidence for a photosensitised oxidation. In a photo initiated autoxidation, radicals would have been generated which would have been expected to react in the dark as well.

In photosensitised oxidations the wavelength of the light source can play an important role. In the case of chlorophylls, emission of light of wavelengths around 400 nm (yellow-green) and 650 nm (blue-green) can be crucial, since the absorption maxima of these natural sensitisers are at 430 nm and 662 nm for chlorophyll a and at 453 nm and 642 nm for chlorophyll b, respectively. According to *Thron et al.* (2001) wavelengths around 400 nm have a greater impact on photoreactions sensitised by chlorophyll derivatives than longer wavelengths. To assess the influence of the illumination, two types of lamps were used (see Fig. 9 in chapter 3.5.2 and Fig. 10 in chapter 3.5.3). The sodium vapour-high pressure lamp (40‘000 lx) emitted much less light in the range of the absorption maxima of the chlorophylls at the lower wavelength range compared to the fluorescent tube (3’200 lx). Thus a less pronounced excitation of the sensitiser in the green tea could be expected. The formation of the aroma compounds to a lesser extent in the experiment with the sodium vapour-high pressure lamp could therefore also be due to the emission spectrum of the lamp.

As discussed in chapter 4.3.1, 2,3-butanedione, 2,3-octanedione and HMND are products obtained from MND by a photooxidative process. Thus, a decrease of MND during a longer period of light exposure and a concurrent increase of the other diones was expected to occur in the light exposure experiments with green tea as well. As shown in Fig. 24 C, this was the case. The amount of 2,3-octanedione steadily increased during the experiments under photooxidative conditions. However, the behaviour of MND, particularly in relation to the formation of HMND, was not as expected. These two β-diketones showed parallel formation curves. No clear cut explanation of this result can be given. The formation of MND and/or HMND could possibly be suppressed from the beginning or in the course of the experiment. It could also be explained through a steady turnover of HMND. Finally MND could constantly be formed from pentyl DiMeF at the same rate as it was oxidised to HMND. More investigations are needed for a better understanding of the complex processes of light induced oxidations in food.

The oxidative behaviour of the pentyl DiMeF of green tea (see Tab. 15, chapter 4.1.2) as precursors of the aroma compounds was investigated by determination of the decrease of the pentyl DiMeF during a prolonged light exposure at 3’200 lx in air. Fig. 25 shows the decrease of the two most abundant pentyl DiMeF during the oxidation procedure, expressed as relative change compared to the sample before light exposure. Both curves showed nearly the same pattern, indicating the oxidation rates to be similar for the two pentyl DiMeF. After 2 days of light exposure, approximately 50 % of the pentyl DiMeF had reacted. As the oxidation process proceeded, the decrease slowed down considerably. At the end of the experiment after 20 days, approximately 10 % of the two pentyl DiMeF were still present. This finding is in contrast to the results described by *Guth and Grosch* (1991), who observed a rapid oxidation of DiMeF(9,5) and DiMeF(11,5) in soy bean oil stored in daylight. After 48 h more than 90 % of the two FFA were degraded. Furthermore, when the oil was exposed to fluorescent light, the two pentyl diMeF disappeared rapidly and were not detectable anymore after 25 h. Butter was rapidly oxidised as well. Only 10 % of the initial concentration of FFA was found after 48 h illumination with fluorescent light (*Guth and Grosch*, 1992). In model experiments by *Yurawecz et al.* (1997) and by *Sehat et al.* (1998), who performed thermical oxidations with F(8,6), the half-life time of this FFA was 35 h.

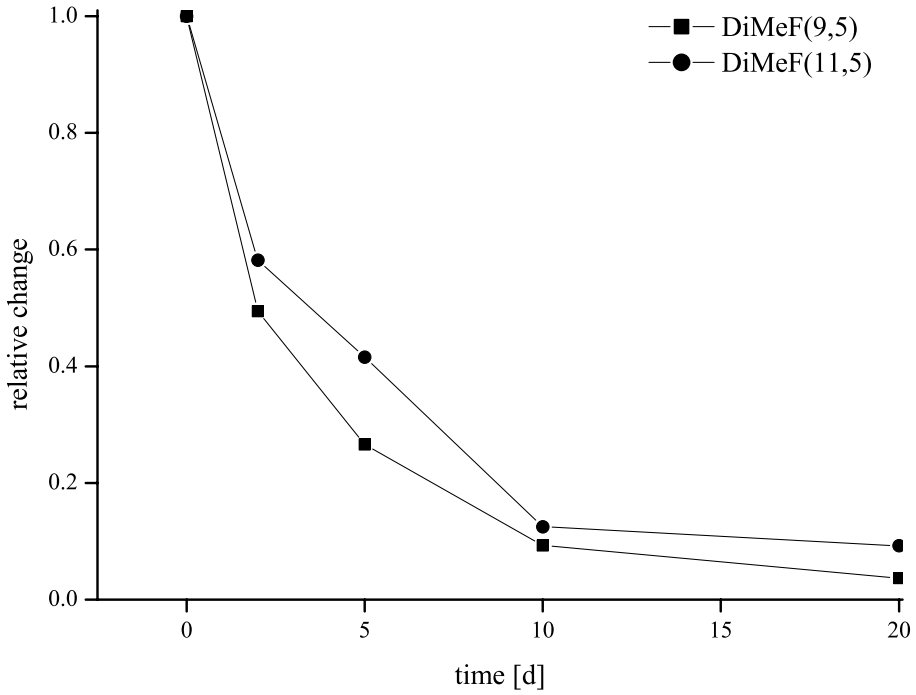


Figure 25: Decrease of the two main pentyl DiMeF in green tea during light exposure (3‘200 lx, air)

The differences between the results obtained with green tea and those with other plant and animal materials as well as pure FFA could be explained by the presence of substances in green tea which quench the action of the photosensitiser. Furthermore, the slower oxidation could also be due to a complicated penetration of light into the dried plant material. A residual amount of pentyl DiMeF after 20 days of light exposure corresponds well to the formation rates of the two bovolides. The steady increase of these two aroma compounds (Fig. 23 B and C) can only be explained if sufficient amounts of the corresponding pentyl DiMeF are available as precursors during the whole duration of the light exposure experiment. The observed decrease of the pentyl DiMeF in green tea further supports the statement regarding a steady turnover of MND during the photooxidative process.

The importance of single aroma compounds for the overall aroma of a food can be roughly estimated by consideration of their thresholds. Tab. 24 shows the odour

thresholds in water of the aroma compounds formed by photooxidation of FFA in green tea.

Table 24: Odour thresholds of aroma active furan fatty acid photooxidative degradation products

aroma compound	threshold in water, orthonasal [µg/kg]	reference
pentanal	12	*Buttery et al.* (1988)
2,3-butanedione	15	*Blank et al.* (1991)
2,3-octanedione	110	*Sigrist et al.* (2000)
MND	0.03	*Masanetz and Grosch* (1998)
HMND	-[1]	-
bovolide	2.0[2]	*Pompizzi* (1999)
dihydrobovolide	500[2]	*Pompizzi* (1999)

1. no data available
2. determined in an oil phase

The thresholds vary in a broad range from very low values (MND) to high values (dihydrobovolide). Based on the thresholds, it can be concluded that MND is the most important of the seven investigated aroma compounds, followed by bovolide, pentanal and 2,3-butanedione. The high threshold of dihydrobovolide indicates that this substance is not important for the overall aroma of green tea exposed to light. The OAV (amount of aroma compound in relation to its threshold) would help to estimate the relevance of the single aroma compounds and a possible change of the overall aroma during light exposure. Unfortunately no OAV could be established, because no absolute amounts of the aroma compounds could be determined. However, since the threshold for MND is 65 times lower as for bovolide and 400 times lower as for pentanal, the other compounds would have to increase much more during light exposure to overcome the OAV of MND.

Once MND has been formed in green tea, further influence of light is not expected to cause relevant changes in the flavour by the investigated aroma compounds.

4.5 Formation of furan fatty acid photooxidative degradation products in dried green herbs

Dried herbs and vegetables were exposed to light at 4‘500 lx in air for four days to induce photooxidation as described in chapter 3.5. In Tab. 25 the effect of light on the pentyl DiMeF present in the investigated herbs and vegetables is shown. The results are expressed as peak area ratio (R_{MS}) to the internal standard ethyl decanoate for the seven aroma compounds. The investigated herbs and vegetables have been shown to be differently susceptible to light exposure. Tarragon, chervil and chive exhibited the most significant changes after light exposure, whereas onions were hardly affected at all. Pentanal, 2,3-butanedione, 2,3-octanedione and the two bovolides were already present before light exposure (except for dihydrobovolide in savory and onion). This result suggests that a slight oxidative change of the samples occurred already before light exposure. In most cases the above mentioned aroma compounds increased during light exposure, thus indicating an oxidation process to take place. The comparatively high formation of pentanal was obviously not only due to the oxidation of pentyl DiMeF. Pentanal is known to be a minor oxidation product of some fatty acids as well (e.g. *Frankel,* 1998). In basil, savory and onion no MND and HMND could be detected even after light exposure. In tarragon, dill, chive and leek very small amounts were found, whereas in chervil, these compounds increased 3-4 times during light exposure. These results can be explained by the contents in pentyl DiMeF of the investigated samples, as shown in Tab. 15 (chapter 4.1.2). Basil, savory and onion are very poor in pentyl DiMeF. Assuming that a part of the formed MND reacted also to HMND, the amounts of these aroma compounds could be below the detection limit in these samples. Yet, the significant increase of MND and HMND in chervil after light exposure agrees well with the comparatively high amounts of DiMeF(9,5) and DiMeF(11,5) present in this herb. One additional reason for the low amounts of MND and HMND in most of the herbs and vegetables has been given by *Guth and Grosch* (1991). In a model experiment, these authors showed that only 1.3 % of the methyl ester of DiMeF(9,5) was converted into MND by photooxidation.

Table 25: Relative concentrations of aroma compounds (expressed as R_{MS}) in different herbs and vegetables before and after light exposure

sample	light exposure	pentanal RI = 986	2,3-butanedione RI = 997	2,3-octanedione RI = 1326	MND RI = 1742	HMND RI = 1726	bovolide RI = 2164	dihydrobovolide RI = 2192
tarragon	–[1]	1	2	tr.[2]	n.d.[3]	n.d.	1	1
	+[4]	9	4	1	1	2	7	1
basil	–	1	1	tr.	n.d.	n.d.	3	4
	+	3	2	1	n.d.	n.d.	4	4
savory	–	1	1	tr.	n.d.	n.d.	1	n.d.
	+	2	1	tr.	n.d.	n.d.	2	n.d.
chervil	–	4	5	1	n.d.	1	4	3
	+	20	6	4	4	3	20	5
dill	–	1	1	tr.	n.d.	tr.	1	1
	+	7	1	1	tr.	tr.	4	1
chive	–	6	4	1	tr.	1	2	tr.
	+	14	4	6	1	2	7	1
onion	–	tr.	tr.	tr.	n.d.	n.d.	tr.	n.d.
	+	tr.	tr.	tr.	n.d.	n.d.	tr.	n.d.
leek	–	1	1	tr.	n.d.	n.d.	1	1
	+	3	1	tr.	tr.	n.d.	2	1

1. before light exposure 2. traces 3. not detected 4. after 4 d at 4‘500 lx in air

In addition to the FFA, other constituents have to be taken into account by interpreting the different behaviour of the investigated herbs and vegetables. The presence of e.g. quenchers and sensitisers can influence the photooxidation process in different ways. Further experiments with edible roots and leaves of plants should be carried out to investigate the influence of plant constituents on the formation of the aroma compounds derived from FFA.

5 CONCLUSION AND OUTLOOK

In previously performed model experiments DiMeF were identified as precursors of the aroma compounds pentanal, 2,3-butanedione, 2,3-octanedione, MND, bovolide and dihydrobovolide formed by oxidative degradation. In this work, the behaviour of DiMeF and the aroma compounds under light exposure in complex food systems was investigated. Green tea, tarragon, basil, savory, dill, chervil, chive, onion and leek were taken as representatives for plant material used as foodstuff. The knowledge obtained by the model experiments could be confirmed and extended.

The sample preparation was the most critical step in the analysis of the aroma compounds, since the method for the isolation of the volatile substances from the non-volatile plant material can strongly influence the result. An adapted micro-SDE method showed to be suitable for the present work. The method turned out to be simple and reproducible. Acceptable recoveries were obtained and no artefacts were formed. Additionally, no concentration step of the isolate was necessary for on column GC analyses. The use of three ISTD gave important information about the reproducibility of the micro-SDE process and allowed to obtain concise results without performing replicate analyses.

Beside the analysis of the aroma compounds, the analysis of the FFA in green tea and the dried green herbs and vegetables was a matter of interest. The GC-MS/MS technique was applied for the first time in the analysis of FFA. Ion trap GC-MS/MS allowed a fast and sensitive identification of the different classes of FFA in the methyl ester extract of plant lipids. To our knowledge, the occurrence of different classes of FFA in tarragon, basil, savory, chervil, dill, onion and leek was reported here for the first time.

In contrast to the model experiments, the investigations with the food samples were complicated by many factors that could not be influenced or remained unknown. The following parameters were recognised to exert influence on the formation and the behaviour of the aroma active FFA degradation products:

Composition of the sample

The amount of the aroma compounds was dependent on the amount and the oxidation rate of the precursor DiMeF present in the samples. Other constituents such as sensitisers, quenchers and water were considered to significantly contribute to the formation of the aroma compounds as well, but were not investigated.

Photooxidative conditions

The formation of the aroma compounds was induced by light exposure in all the investigated samples. All aroma compounds increased during the two first days of light exposure. Light exposure in oxygen atmosphere resulted in higher amounts of the aroma compounds than light exposure in air. The amount of the aroma compounds were affected by the light sources, which differed in the illuminance and the light emission spectrum.

Stability of the aroma compounds

MND and 2,3-octanedione were shown in model experiments to react to other aroma compounds under photooxidative conditions. The aroma compounds which derived from 2,3-octanedione were pentanal, acetic acid and hexanoic acid. The aroma compounds which derived from MND were HMND, 2,3-butanedione, 2,3-octanedione, acetic acid and hexanoic acid. HMND was identified for the first time in this work and was also detected in the food samples.

Other factors which could have influenced the formation and the behaviour of the aroma active FFA degradation products could not be considered in this study. Further investigations with model experiments are needed to gain a better understanding of light induced formation and changes of the investigated aroma compounds.

With the relative amounts of the aroma active FFA degradation products, no final conclusion could be drawn on their relevance in the food samples. Sensory analyses should be conducted to investigate the contribution of the aroma compounds to the flavour or off-flavour of green tea and the investigated herbs and vegetables. The aroma

compounds investigated should be considered together with all other aroma active volatiles in the samples. AEDA in combination with the calculation of OAV would help to determine the most potent aroma compounds in each sample. Finally, the instrumental analyses, in particular olfactory analyses, should be confirmed by sensory investigations on the foodstuff itself.

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